

The diagram illustrates the Cre-loxP recombination system for subcloning. It shows three main stages:

- Top Stage:** Two circular DNA molecules are shown. On the left is the **INSERT DONOR** (labeled **B (CLONING VECTOR)**), which contains an **INSERT** (labeled **A**) flanked by two loxP sites (represented by small squares). On the right is the **VECTOR DONOR** (labeled **D (SUBCLONING VECTOR)**), which contains a **REPRESSION CASSETTE** (labeled **C**) flanked by two loxP sites.
- Middle Stage:** The two molecules undergo recombination, catalyzed by the enzyme **RECOMBINASE** (represented by a small square). This results in a single circular DNA molecule labeled **COINTEGRATE**. This molecule contains both the insert **A** and the repression cassette **C**, each flanked by loxP sites.
- Bottom Stage:** A second round of recombination is performed, catalyzed by **RECOMBINASE** (represented by a small circle). This step removes the repression cassette **C**, resulting in two products:
 - SUBCLONE = PRODUCT** (labeled **D**): A circular DNA molecule containing the insert **A** flanked by loxP sites.
 - BYPRODUCT** (labeled **B**): A circular DNA molecule containing the repression cassette **C** flanked by loxP sites.

FIG. 1

10005075-120704

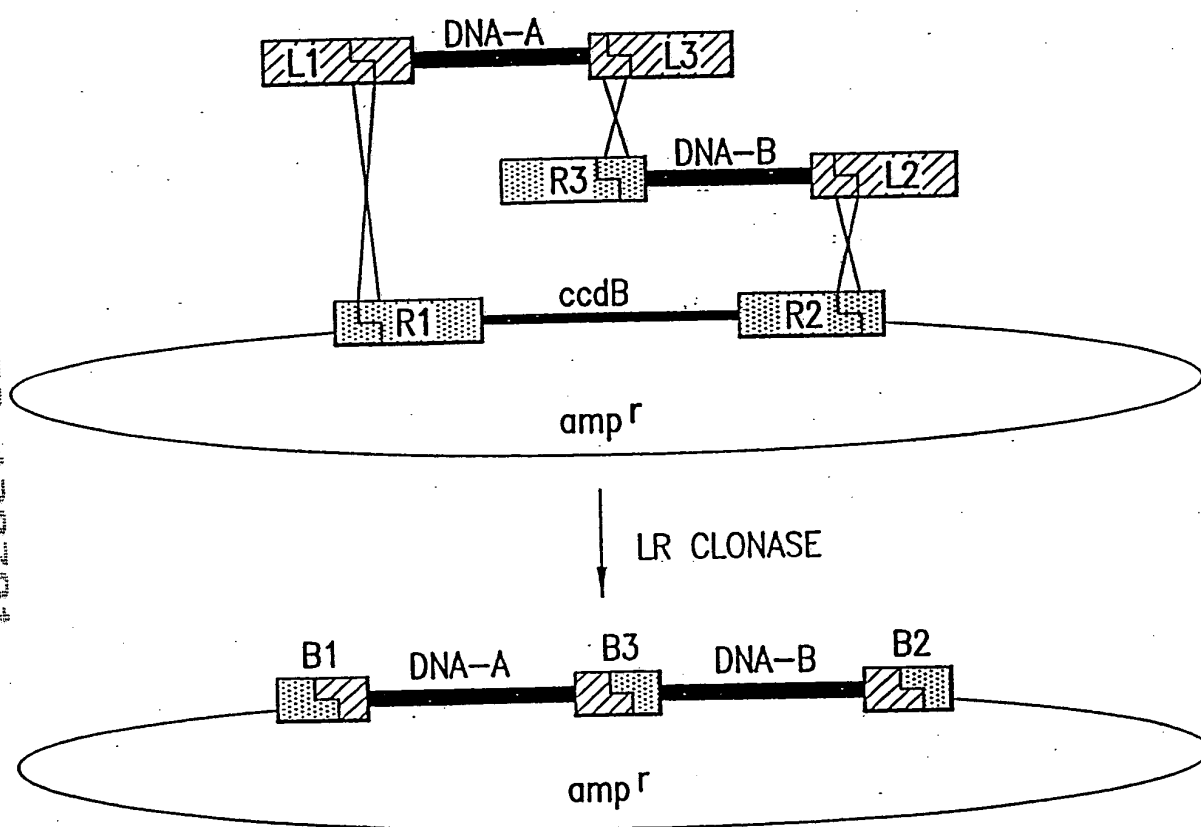


FIG.2

FIG. 3

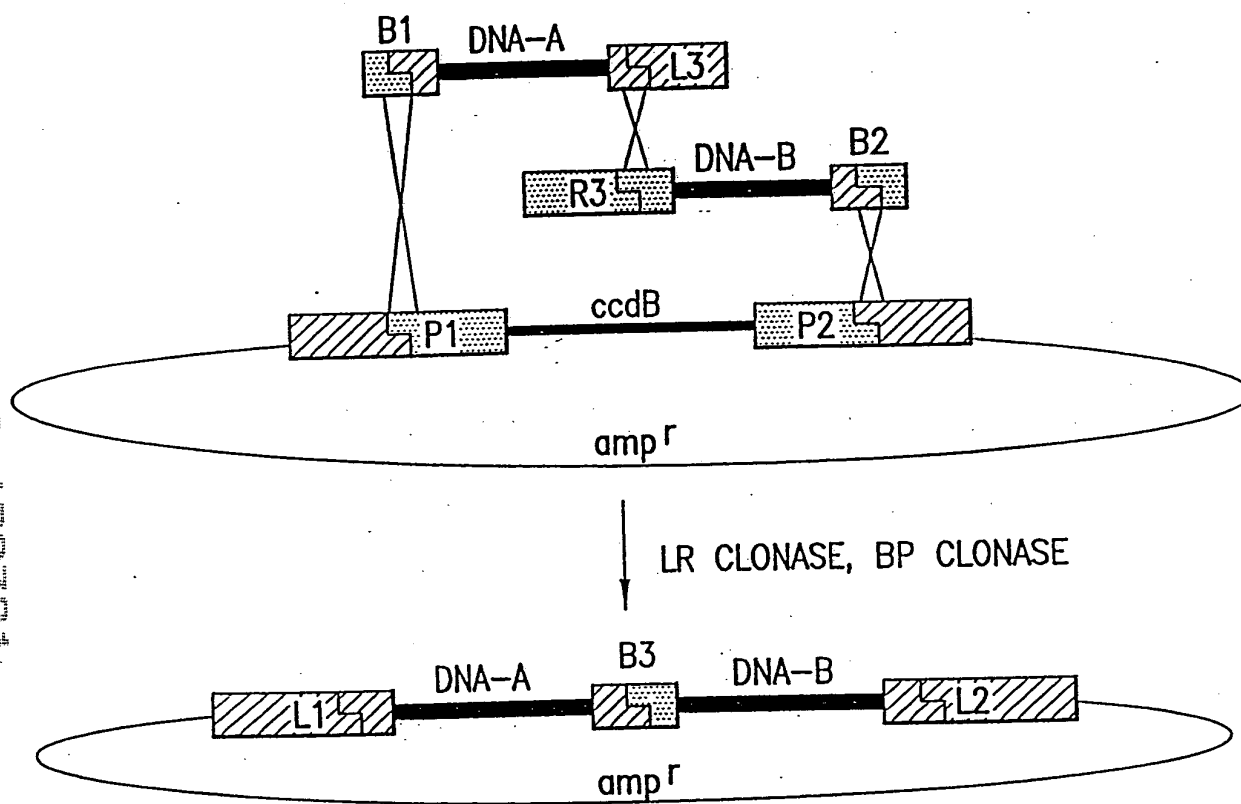


FIG.3

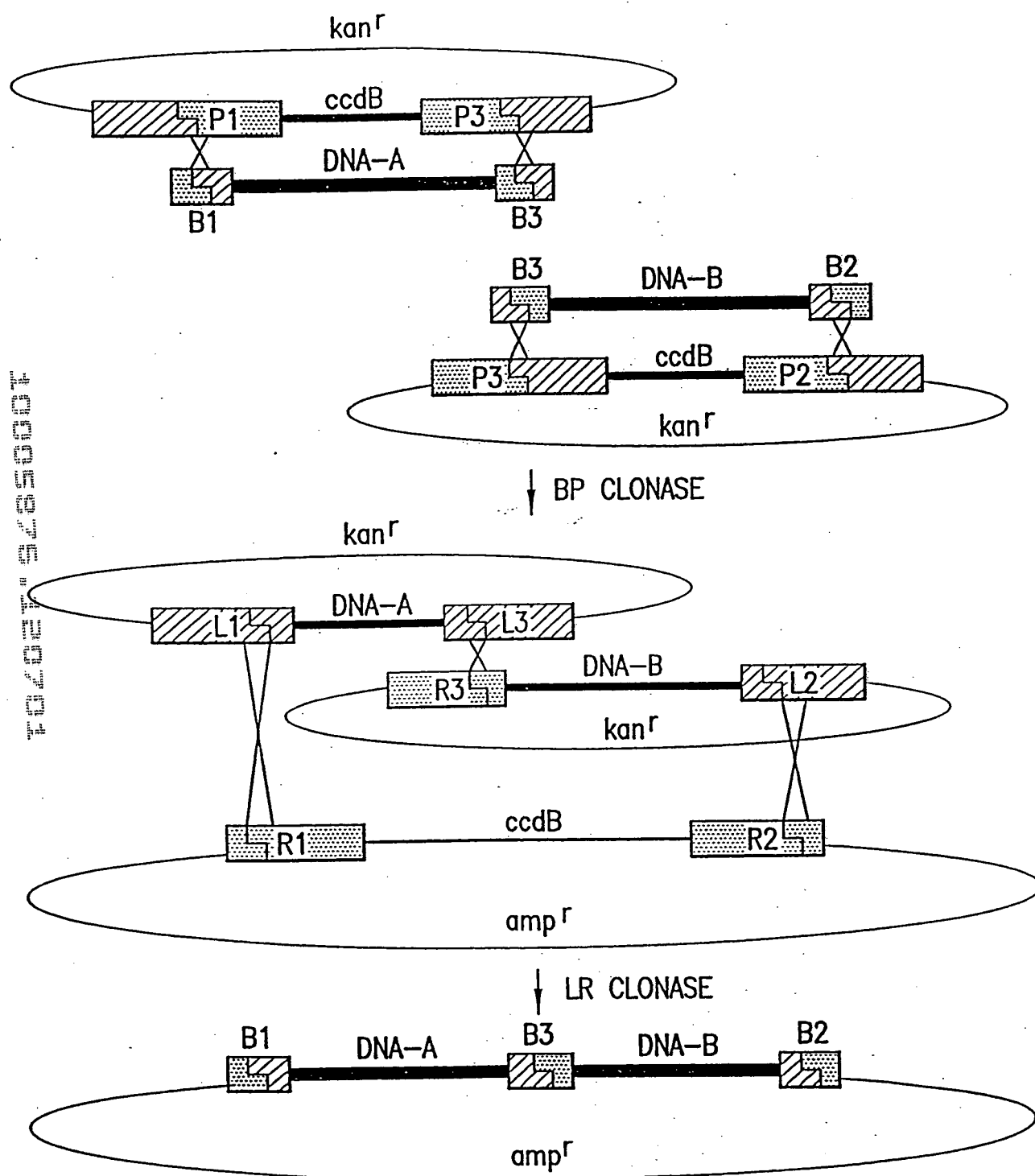


FIG.4

10005976-120704

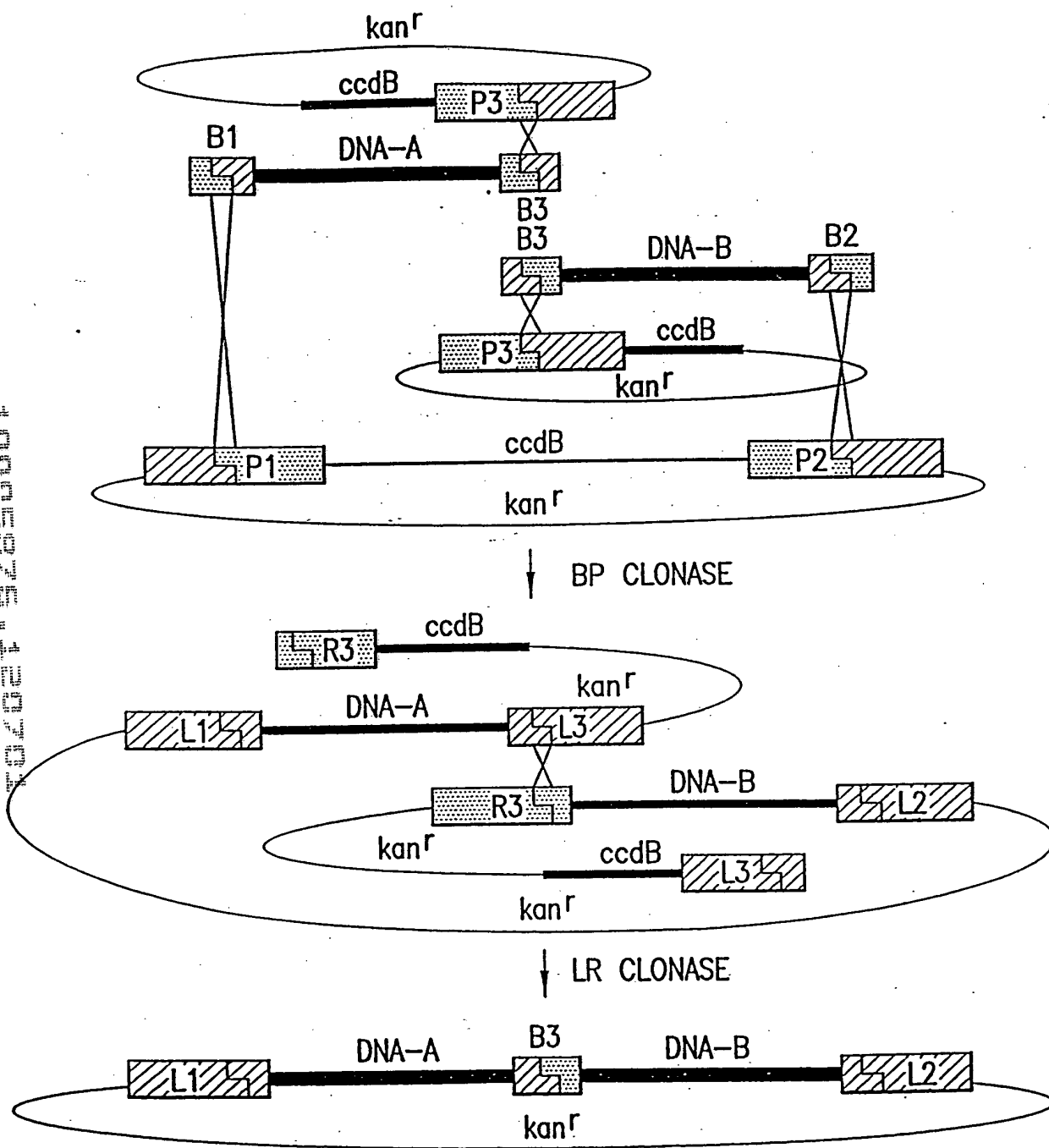


FIG.5

10005076 420704
T022T 9205000T

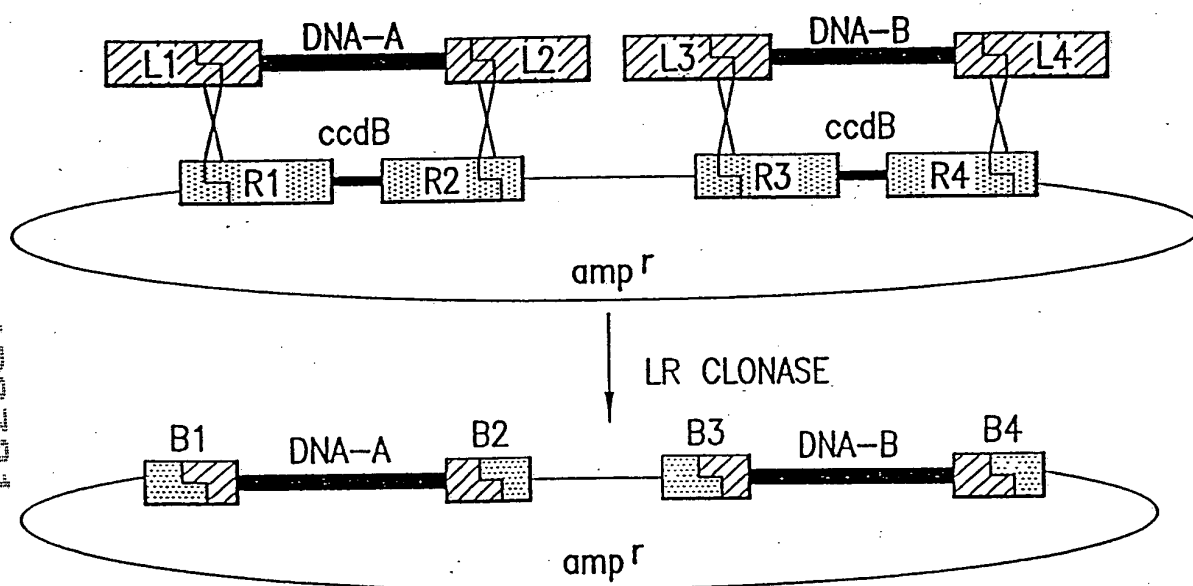


FIG.6

Target: 5'GGGTTT

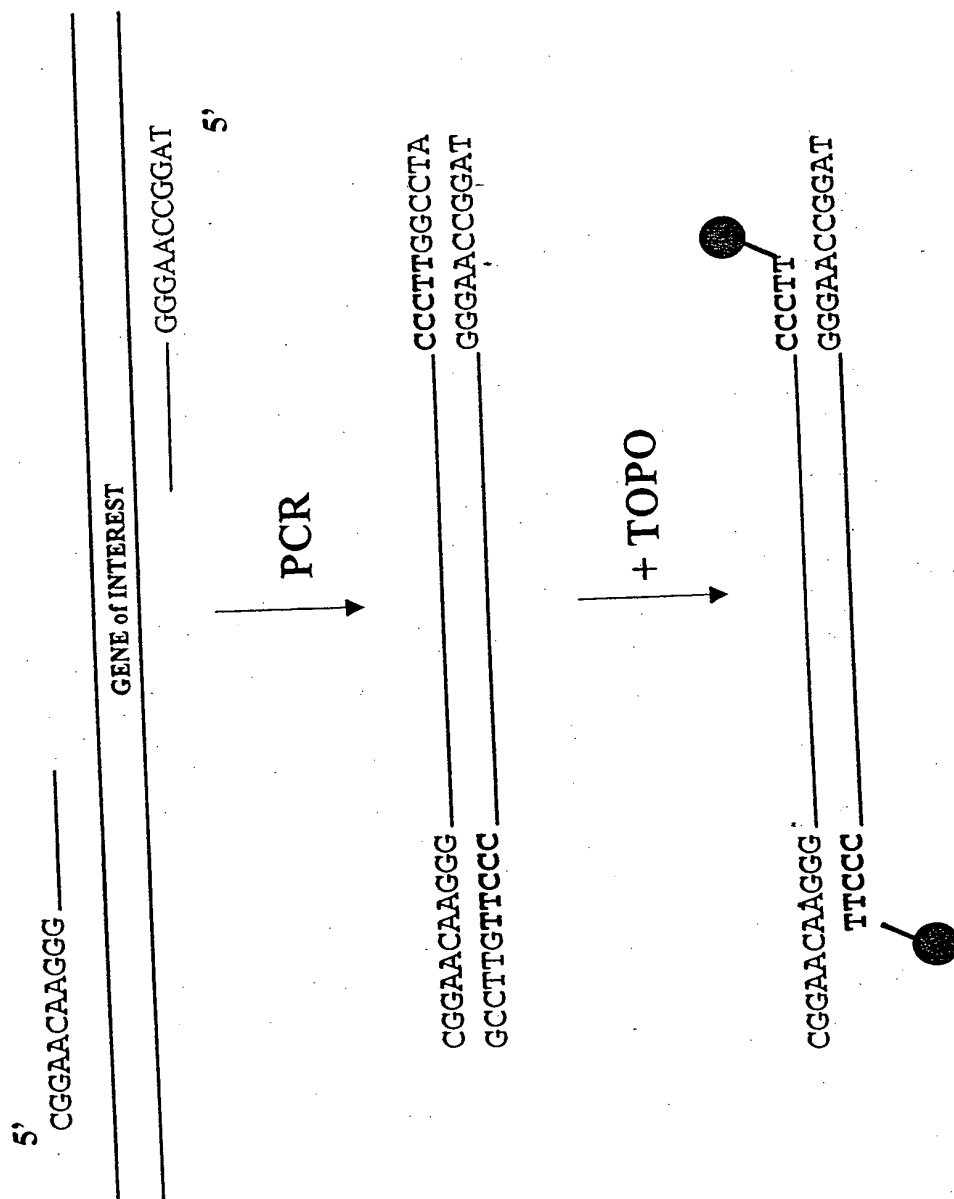


FIGURE 8A

TOPO-2" 3/25/00

CGGAACAAGG _____ CCCTT
TTCCC _____ GGAACCGGAT

Element 2

GGCCATAAGG _____
TTCCC _____ P2

+TOPO-adapted
elements

P1 _____ CCCTT
_____ GGAAGCCTTG

Element 1

CCCTTGGCCATAAGG _____
GGGAACCGGATTTCCC _____

PCR with P1/P2

FIGURE 8B

FOCCT" 9450000T

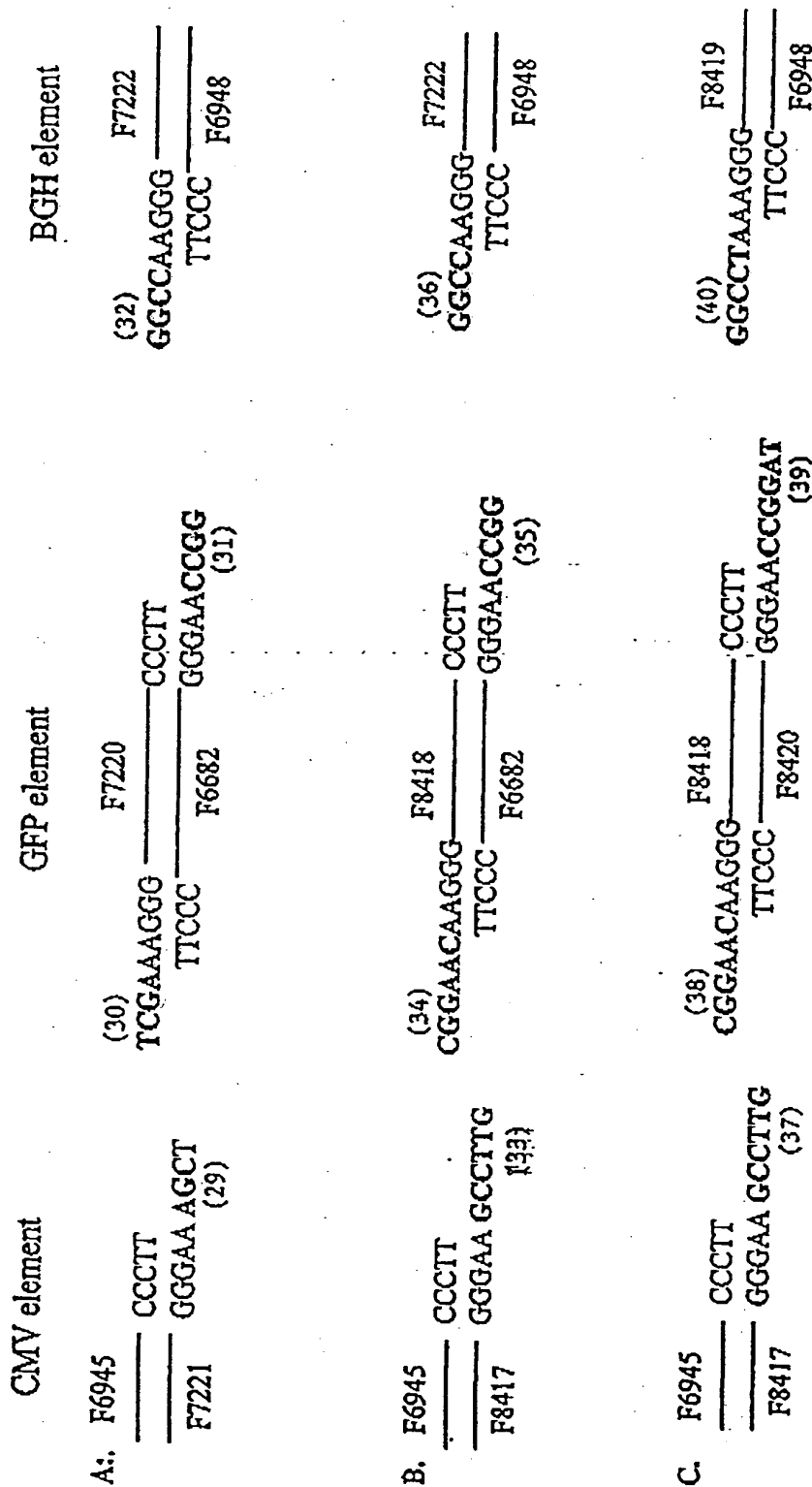


Figure 9A-C

TABLE 1

Table 1

SEQ ID NO:

Primer name	F#	Sequence (5' → 3')
MTH1	10779	TATGTATCATACATACGATTAGGT
MTH2	10780	ACCGCCTCTCCCGCGGTT
GAL4/2	12667	GTTCCGAAGGGGGGATACAGTCAACTGCTTTG
MTH5	12505	TTGGCCAAGGGTATCTAGAAGCTTCTGCAGACGCGT
VP16/2	12668	GTTCCGAAGGGCCACCGTACTCGTCAATTCGAAG
SV40pA	12016	GGCCAAAGGGAACTGTTTATTCAGCTTATAATG
SV40pA	561	CTCTGACTTGAGCGTCGATTT
p53/2	12669	CGGAACAAGGGGAATCCCTGTCCACCGAGACC
SVT/2	12670	CGGAACAAGGGGAATCCCGGGGATCTGGAATTC
CMV/2	7221	TCGAAAGGGTCGAGGTCGACCTGCAGCTG
CMV1	6945	AATTCACATTGATTATTGAGTAGTTA
GFP-Xhof	7220	TCGAAAGGGTAATGGCCAGCAAGGAGAG
GFP-Noir	6582	GGCCAAGGGTTTGTAGAGCTCATCCAT
BGH/2	7222	GGCCAAGGGTCTGAATGGGGCCGCATAGT
BGHr	6948	AAGCCATAGAGCCCGGGCCA
CMV/3	8417	GTTCCGAAGGGTCGAGTCCGACCTGCAGCTG
GFP/3	8418	CGGAACAAGGGATGGCCAGCAAGGAGAAG
GFP/3	8420	TAGGCCAAGGGTTTGTAGAGCTCATCG
BGH/3	8419	GGCCTAAAGGGTGAATGGGGCCGCATAGT
T7top	9304	GAAGGAGTAATACGACTCACT
T7bottom	9305	GTTCCGAAGGGCCCATGGTGGCTCCCTATAGTGAGTGGTACTCTTC
T7amp	9306	GAAGGAGTAATACGACTCACT
T3top	9661	GGCCTAAAGGGTCCCTTTAGTGAGGGTTAATGCCGCG
T3bottom	9662	GGCGGCAATTAAACCTCACTAAAGGGACCCCTTAGGGC
lacZ/2	10532	CGGAACAAGGGGATGATAGATCCCGTGGTTTACA
lacZ1k2	10770	TAGGCCAAGGGGAGACCATTTTCAATCCGCACCT
lacZ2k2	10771	TAGGCCAAGGGGAGGACCTTACCGCTTGCCA
lacZ3k2	10772	TAGGCCAAGGGTTTGACACACAGACCAACTGGTA

Figure 9D

A.

Sample #	GAL4+pa	VP16+pa	pGene/lacZ	GAL4+p53+pa	VP16+1+pa	p53-VP16
1			0.26 ug	p 0.37 ug	p 0.37 ug	
2			0.4 ug	p 0.3 ug	p 0.3 ug	
3			0.4 ug			p 0.6 ug
4			0.4 ug	10.3 ug	10.3 ug	
5		10.3 ug	0.4 ug	10.3 ug		
6	10.3 ug		0.4 ug		10.3 ug	
7			0.4 ug	4.5 ul PCR	4.5 ul PCR	
8		4.5 ul PCR	0.4 ug	4.5 ul PCR		
9	4.5 ul PCR		0.4 ug		4.5 ul PCR	

B.

Sample #	LacZ activ
1	240000
2	140000
3	1800000
4	1400000
5	54000
6	80000
7	320000
8	12000
9	42000

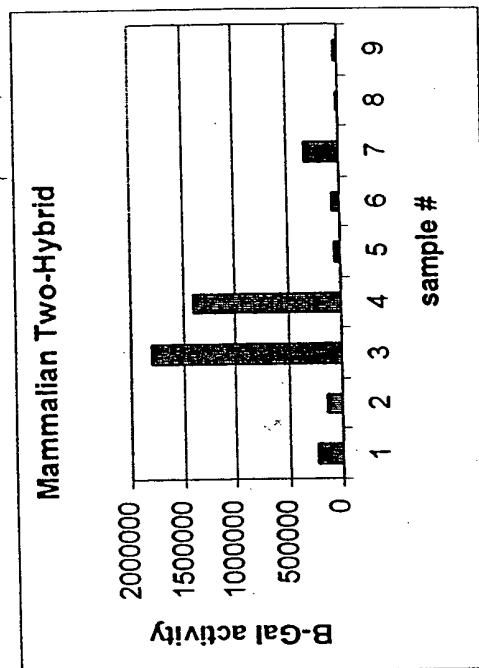


FIGURE 10

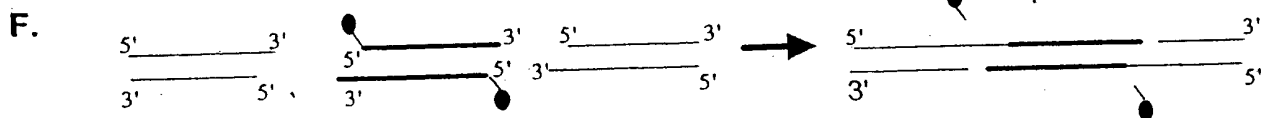
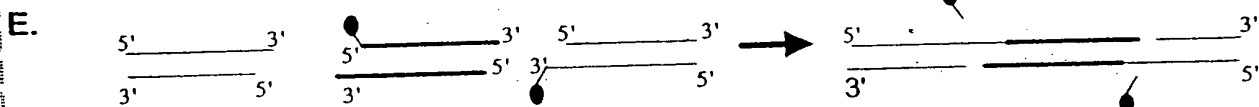
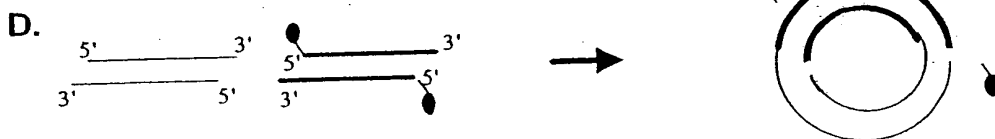
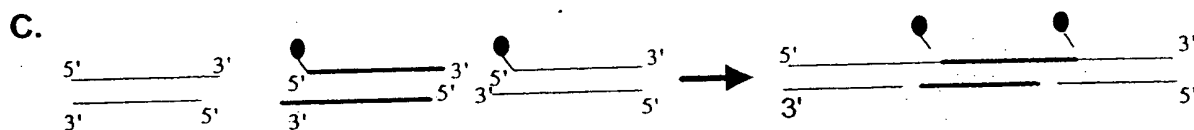
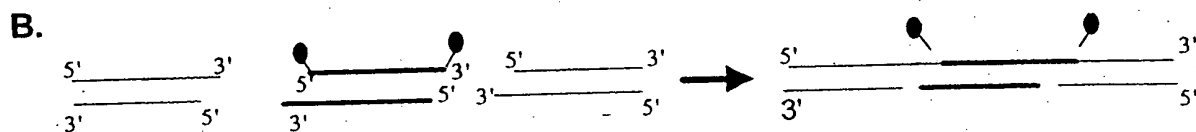
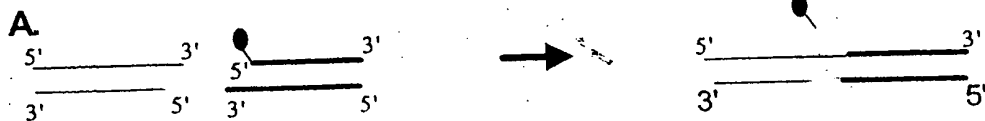
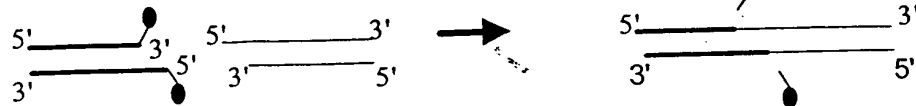
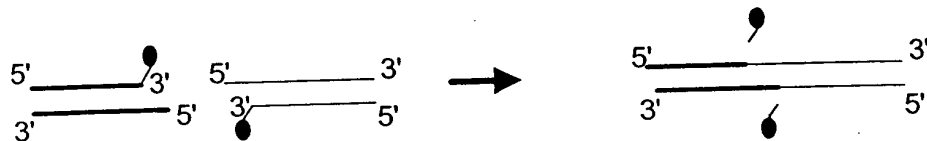


FIGURE 11

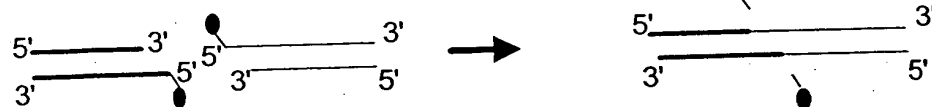
A.



B.



C.



D.

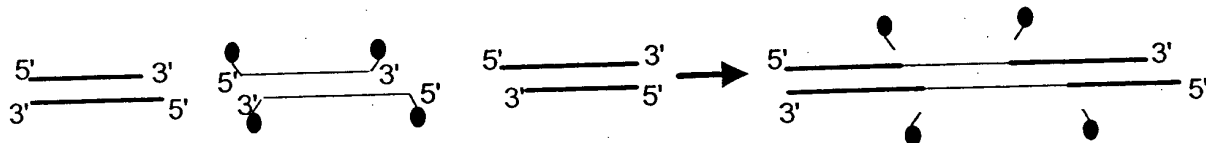


FIGURE 12

10005075 120701
10/20/01 9/26/00

FIGURE 13

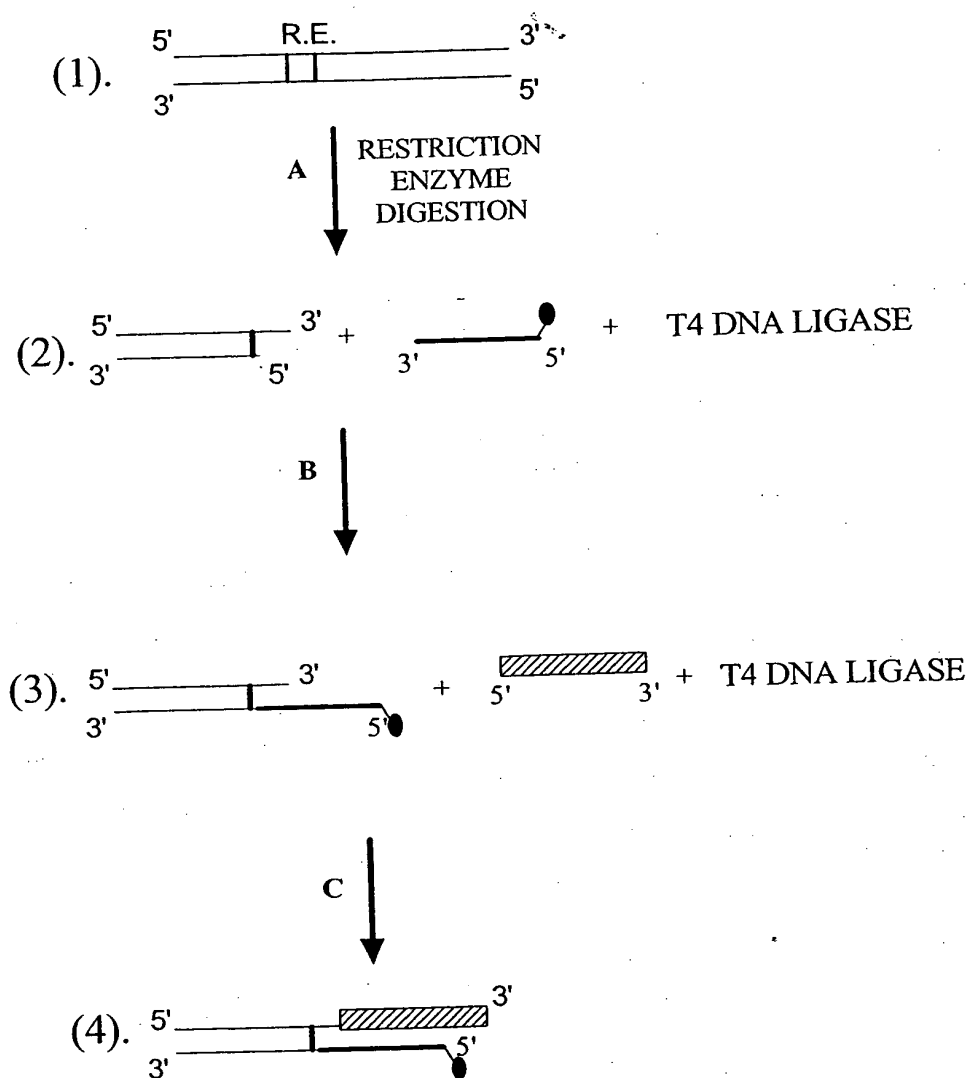
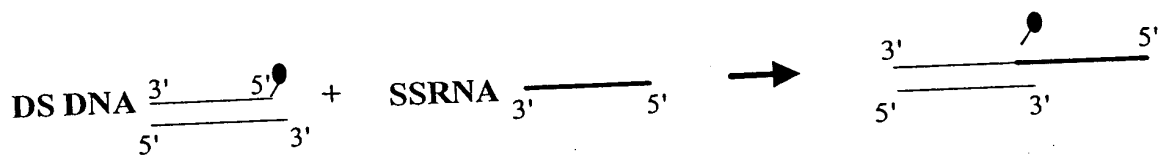
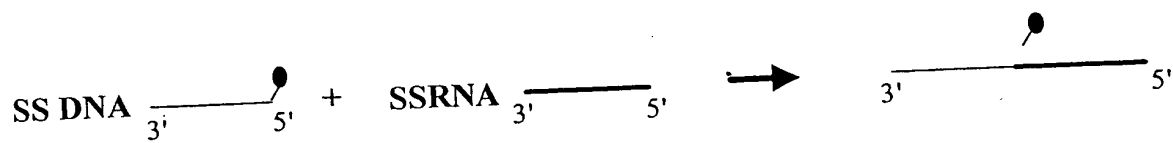
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FIGURE 15



1005376-10704

1005876-12004
1005876-12004

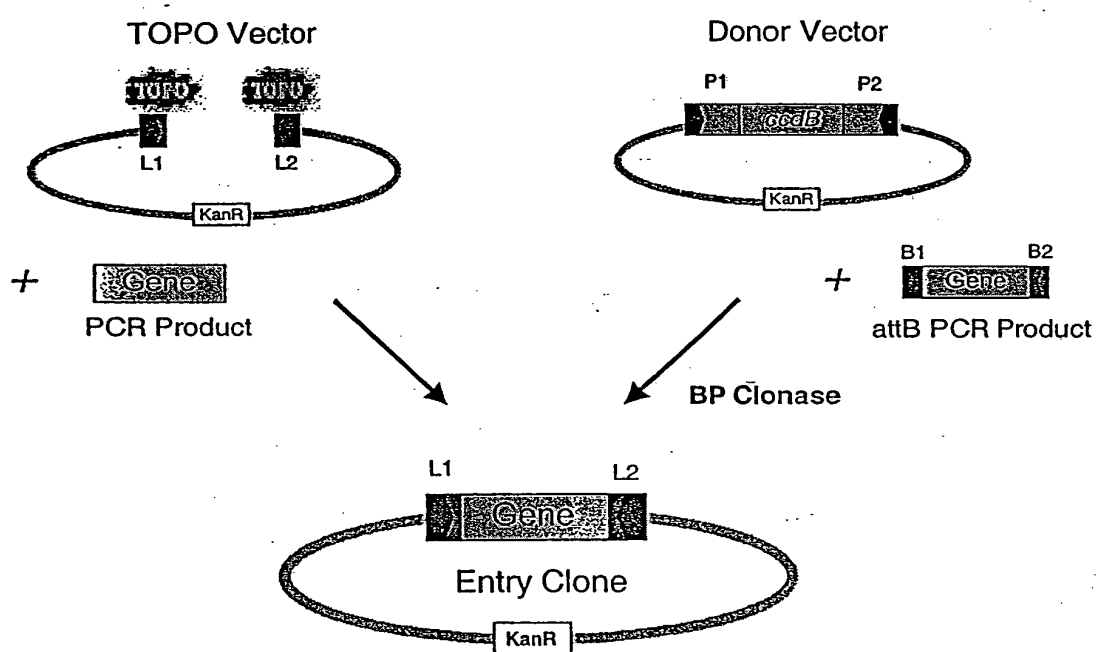


FIGURE 16

405836 4074
545004
545004

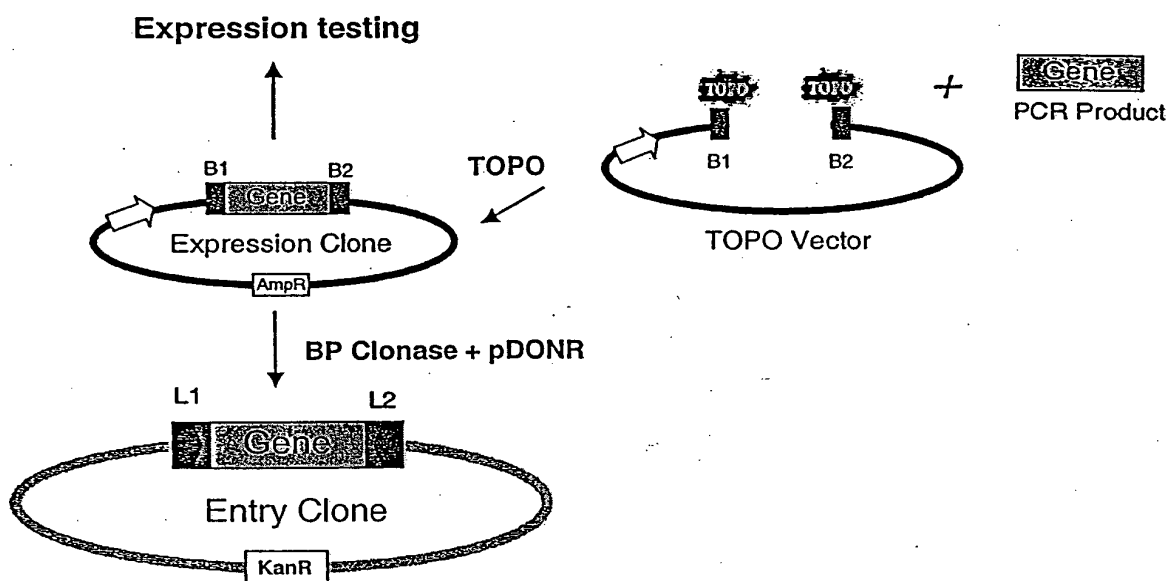


FIGURE 17

MCS for pcDNAGW-DT(sc) and pENTR-DT(sc)

L Y K K A G S A A A G R A D P A F L Y K V
 ...TTG TAC AAA AAA GCA GGC TCC GCG GCC GCC GTA CTC GAG AAA GGG CGC GCC GAC CCA GCT TTC TTG TAC AAA GTG
BsrGI *NotI* *XhoI* *AscI* *BsrGI*
 [A111/B1] [A112/B2]

FIGURE 18

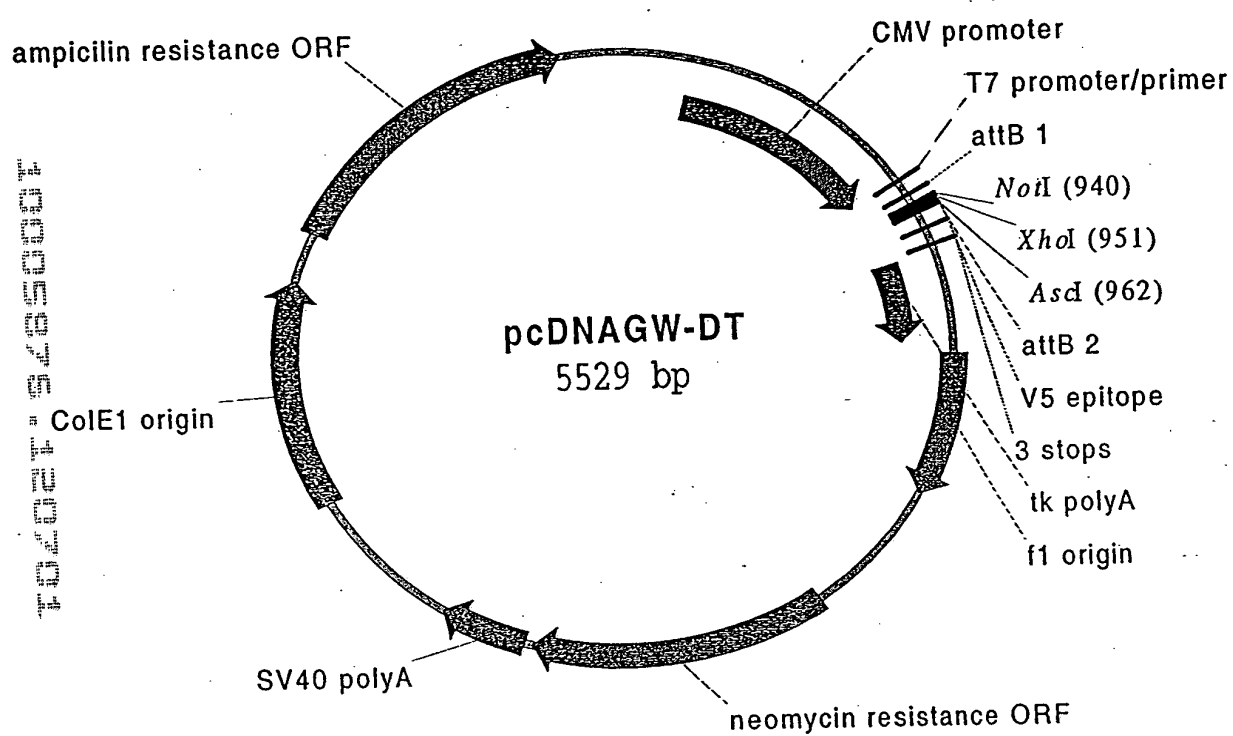


FIGURE 19

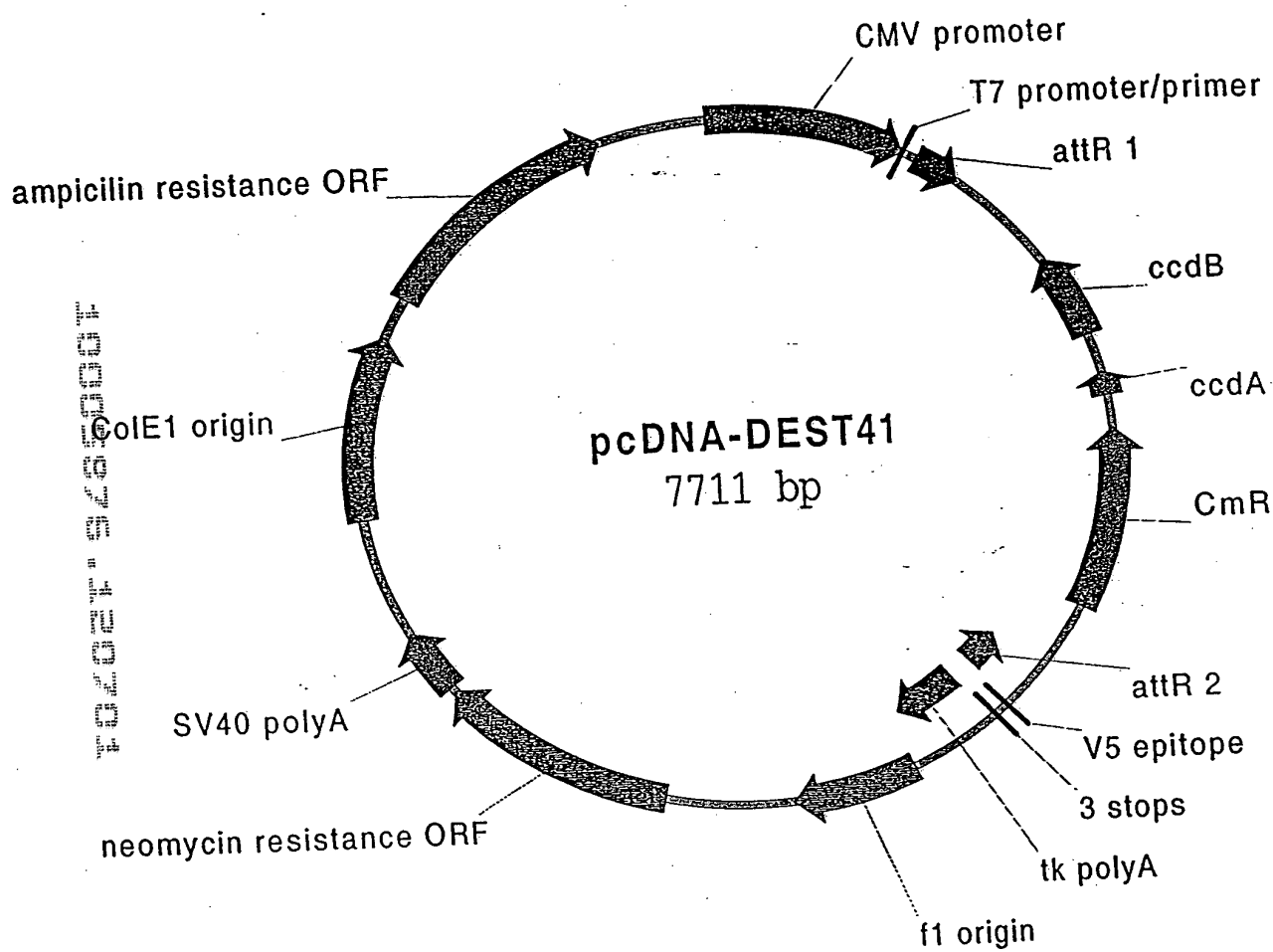


FIGURE 20

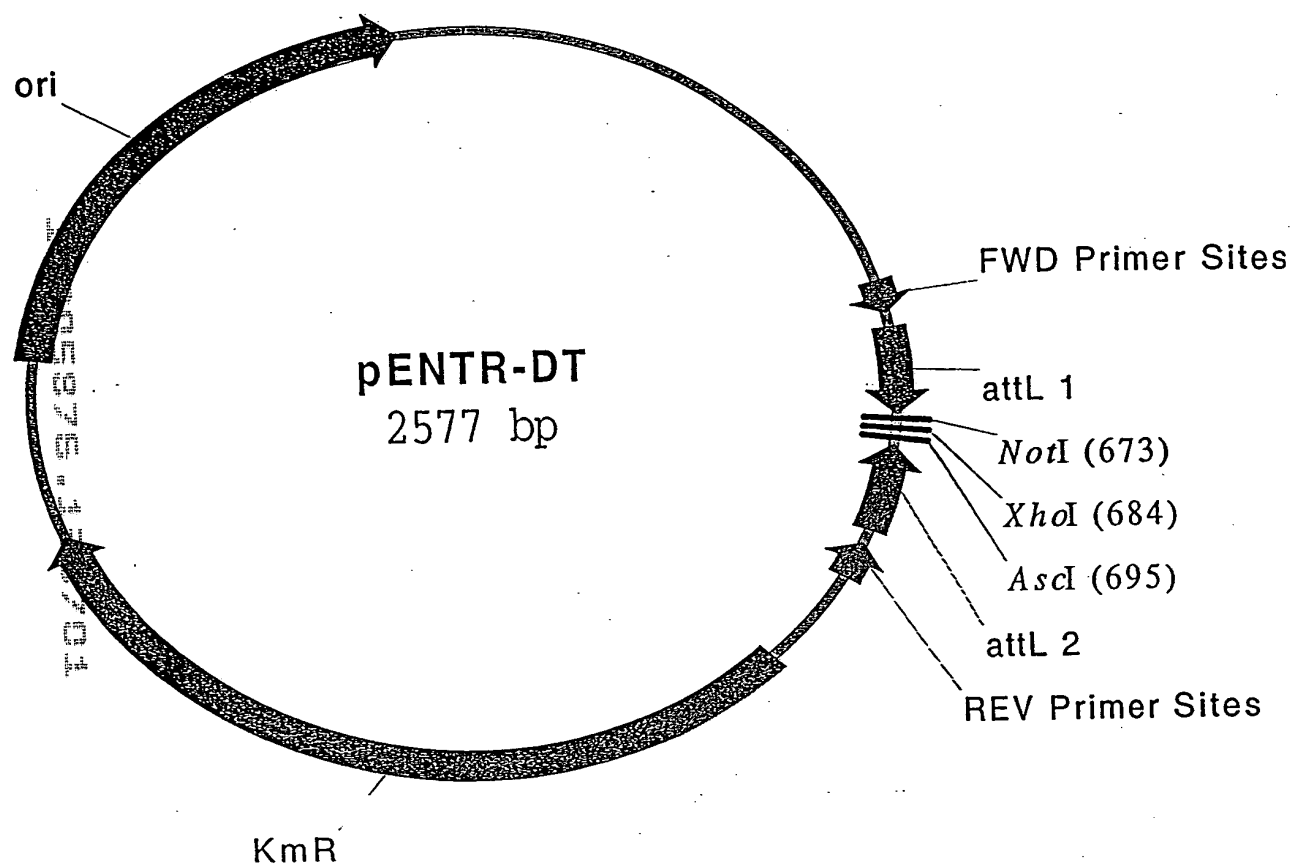


FIGURE 21

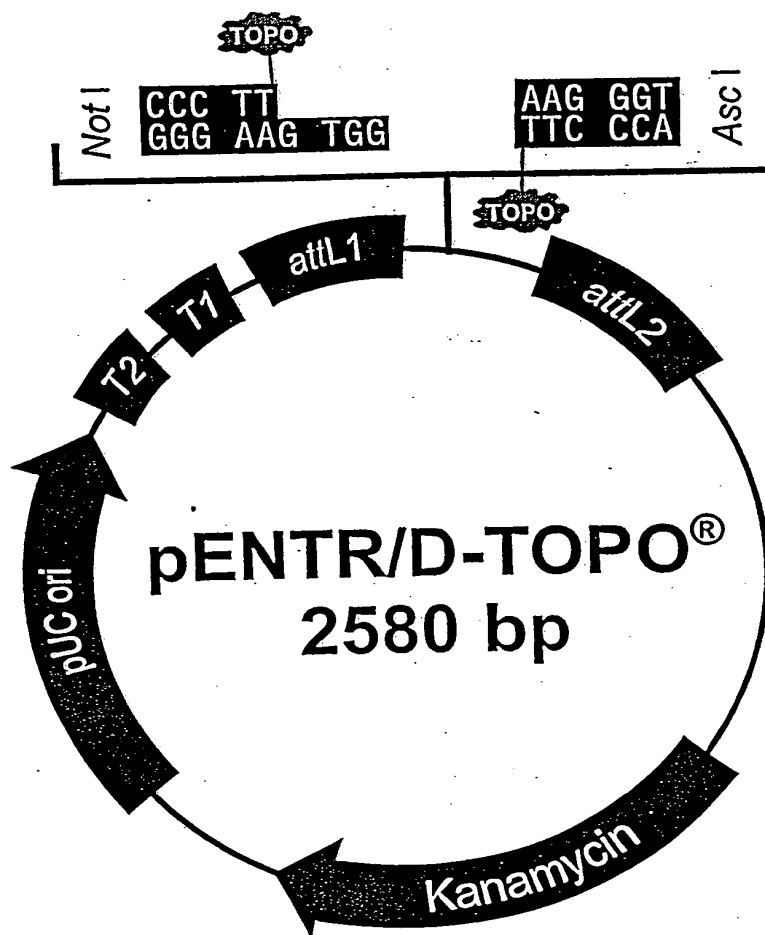


FIGURE 22A

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121	gcgcccata	cgcaaaccgc	ctctccccgc	gcgttgcccg	attcattaat	gcagctggca
181	cgacagggtt	cccgaactga	aagcggggcag	tgagcgcaac	gcaattaata	cgcgctaccgc
241	tagccaggaa	gagttttag	aaacgcaaaa	aggccatccg	tcaggatggc	cttctgctta
301	gtttgatgcc	tggcagttta	tggcggggcg	cctgcccgcg	accctccggg	ccgttgcttc
361	acaacgttca	aatccgctcc	cgggcggttt	gtcctactca	ggagagcggt	caccgacaaa
421	caacagataa	aacgaaaggg	ccagtcttcc	gactgagcct	ttcgttttat	ttgatgcctg
481	gcagttccct	actctcgcgt	taacgctagc	atggatgttt	ttccagtcac	gacgttgtaa
541	aacgacggcc	agtcttaagc	tggggcccca	aataatgatt	ttattttgac	tgatagtgcg
601	ctgttcggtg	caacaaattg	atgagcaatg	cttttttata	atgccaaact	tgtacaaaaa
661	agcaggctcc	gcggccgccc	cttcaccatg	nnnnnnnnna	aggggtgggg	cgccgaccca
721	gctttcttgt	acaaagttgg	cattataaga	aagcatttgt	tatcaatttg	ttgcaacgaa
781	cagggtcacta	tcagtcaaaa	taaaatcatt	atttgccatc	cagctgatat	cccctatagt
841	gagtcgtata	acatggctcat	agctgtttcc	tggcagctct	ggcccgtgtc	tcaaaatctc
901	tgatgtttaca	ttgcacaaga	taaaaatata	tcattcatgaa	caataaaact	gtctgcttac
961	ataaacagta	atacaagggg	tgttatgagc	catattcaac	gggaaacgtc	gaggccgcga
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1081	caatcagggtg	cgacaatcta	tcgctttagt	gggaagcccg	atgcgccaga	gttgtttctg
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1261	tggttactca	ccactgcgat	ccccggaaaa	acagcattcc	aggtattaga	agaatatcct
1321	gattcagggtg	aaaatattgt	tgatgcgctg	gcagtgttcc	tgcgcgggtt	gcattcgatt
1381	cctgttttga	attgtccttt	taacagcgat	cgcgattttc	gtctcgctca	ggcgcaatca
1441	cgaatgaata	acggtttggt	tgatgcgagt	gattttgatg	acgagcgtaa	tggctggcct
1501	gttgaacaag	tctggaaaga	aatgcataaa	cttttgccat	tctcaccgga	ttcagtcgtc
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1621	attgatgttg	gacgagtcgg	aatcgcagac	cgataaccagg	atcttgccat	cctatggaac
1681	tgcctcggtg	agttttctcc	ttcattacag	aaacggcttt	ttcaaaaata	tgggtattgat
1741	aatcctgata	tgaataaatt	gcagtttcat	ttgatgctcg	atgagttttt	ctaatacagaa
1801	ttgggttaatt	ggttgtaaca	ctggcagagc	attacgctga	cttgacggga	cgccgcaagc
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1921	gaaaagatca	aaggatcttc	ttgagatcct	ttttttctgc	gcgtaatctg	ctgcttgcaa
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2101	ccgtagtttag	gccaccactt	caagaactct	gtagcaccgc	ctacatacct	cgctctgcta
2161	atcctgttac	cagtggctgc	tgccagtggc	gataagtcgt	gtcttaccgg	gttggactca
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2341	agcgccacgc	ttcccgaagg	gagaaaggcg	gacaggtatc	cggtaaagcg	cagggtcgga
2401	acaggagagc	gcacgaggga	gcttccaggg	ggaaacgcct	ggatatctta	tagtctgtc
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2521	ctatggaaaa	acgccagcaa	cgcggccttt	ttacggttcc	tggccttttg	ctggcctttt
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FIGURE 22B

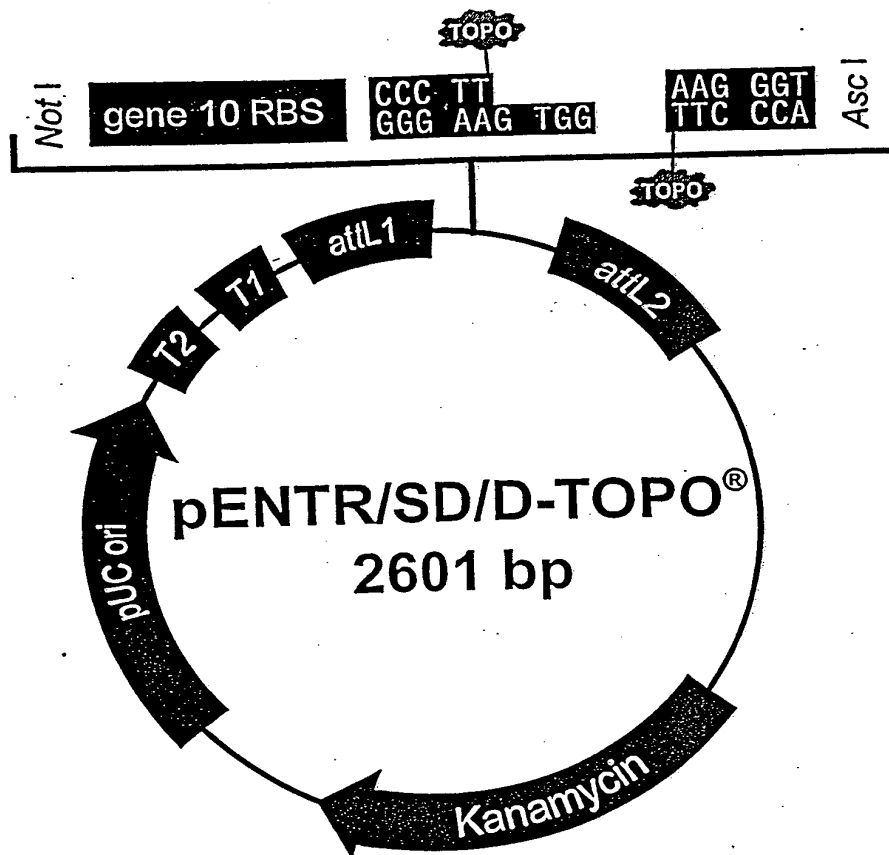


FIGURE 23A

1 ctttcctgcg ttatcccctg attctgtgga taaccgtatt accgcctttg agtgagctga
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121 gcgccaata cgcaaacgc ctctcccgc gcgttgccg attcattaat gcagctggca
181 cgacaggttt cccgactgga aagcgggcag tgagcgcaac gcaattaata cgcgtaccgc
241 tagccaggaa gagttttag aaacgcaaaa aggccatccg tcaggatggc cttctgctta
301 gtttgatgcc tggcagttta tggcgggcgt cctgcccgc accctccggg ccgttgcttc
361 acaacgttca aatccgctcc cggcggattt gtccactca ggagagcgtt caccgacaaa
421 caacagataa aacgaaaggc ccagtcttcc gactgagcct ttcgttttat ttgatgcctg
481 gcagtccct actctcgcgt taacgctagc atggatgttt tccagtcac gacgttgtaa
541 aacgacggcc agtcttaagc tcgggcccc aataatgatt ttattttgac tgatagtac
601 ctgttcgttg caacaaattg atgagcaatg cttttttata atgccaaact tgtacaaaaa
661 agcaggtccc gcggccgcct tgtttaactt taagaaggag cccttcaccn nnnnnaaggg
721 tgggcgcgcc gaccagctt tcttgtaaa agttggcatt ataagaaagc attgcttatc
781 aatttggtgc aacgaacagg tcactatcag tcaaaataaa atcattattt gccatccagc
841 tgatatcccc tatagttagt cgtattacat ggtcatagct gtttcctggc agctctggcc
901 cgtgtctcaa aatctcgtat gttacattgc acaagataaa aatatatcat catgaacaat
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1021 aacgtcgcgg ccgcgattaa attccaacat ggatgctgat ttatatgggt ataaatgggc
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1861 acgggacggc gcaagctcat gacaaaaatc ccttaacgtg agttacgcgt cgttccactg
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2341 gcgtgagcat tgagaaagcg ccacgcttcc cgaagggaga aaggcggaca ggtatccggg
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2461 tctttatagt cctgtcgggt ttcgccacct ctgacttgag cgtcgatttt tgtgatgctc
2521 gtcagggggg cggagcctat ggaaaaacgc cagcaacgcg gcctttttac ggttctctggc
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FIGURE 23B

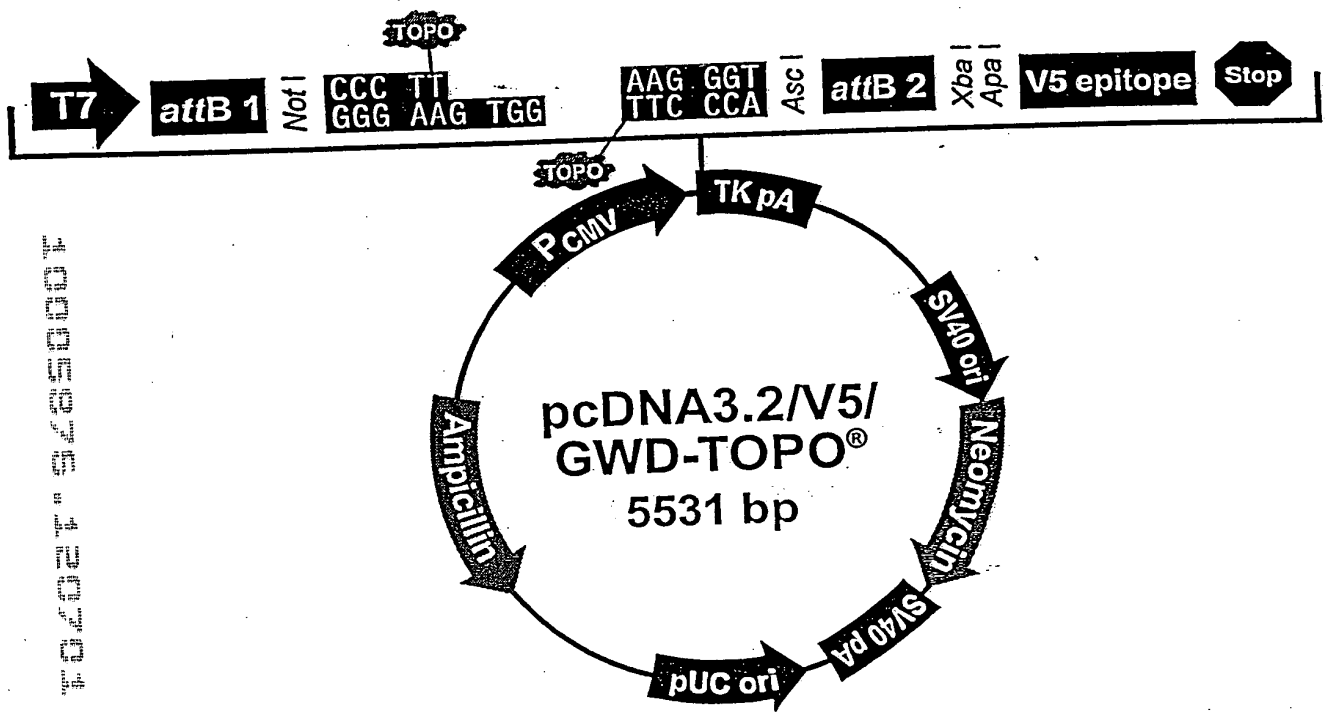


FIGURE 24A

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121 cgagcaaaat ttaagctaca acaaggcaag gcttgaccga caattgcatg aagaatctgc
181 ttagggtagt gcggttttgcg ctgcttcgcg atgtacgggc cagatatacg cgttgacatt
241 gattattgac tagttattaa tagtaatcaa ttacgggggc attagttcat agcccatata
301 tggagttccg cgttacataa cttacggtaa atggcccgcc tggctgaccg cccaacgacc
361 cccgcccatt gacgtcaata atgacgtatg ttcccatagt aacgccaata gggactttcc
421 attgacgtca atgggtggac tatttacggg aaactgccc cttggcagta catcaagtgt
481 atcatatgcc aagtacgccc cctattgacg tcaatgacgg taaatggccc gcctggcatt
541 atgcccagta catgacctta tgggactttc ctacttggca gtacatctac gtattagtca
601 tcgctattac catggtgatg cgggttttggc agtacatcaa tgggctgga tagcggtttg
661 actcacgggg atttccaagt ctccacccc a ttgacgtcaa tgggagtttg ttttggcacc
721 aaaatcaacg ggactttcca aaatgtcgta acaactccgc cccattgacg caaatgggcg
781 ctaggcgtgt acggtgggag gtctatcgaa gcagagctct ctggctaact agagaaccca
841 ctgcttactg gcttatcgaa attaatacga ctactatag ggagacccaa gctggctagt
901 taagctatca acaagtttgt acaaaaaagc aggtcccgcg gccgcccctt caccatgnnn
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1021 ccgcggttcg aaggtaagcc tatccctaac cctctcctcg gtctcgattc tacgcgtacc
1081 ggttagtaat gagtttaaac gggggagggt aactgaaaca cggaaggaga caataccgga
1141 aggaaccgcg gctatgacgg caataaaaag acagaataaa acgcacgggt gttgggtcgt
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1321 gtgaaggccc agggctcgca gccaacgtcg gggcggcagg cctgccata gcagatctgc
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FIGURE 24B

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3301	ctagttgtgg	tttgtccaaa	ctcatcaatg	tatcttatca	tgtctgtata	ccgtcgacct
3361	ctagctagag	cttggcgtaa	tcatggtcac	agctgtttcc	tgtgtgaaat	tgttatccgc
3421	tcacaattcc	acacaacata	cgagccggaa	gcataaagtg	taaagcctgg	gggtgcctaat
3481	gagtgagcta	actcacatta	attgcgttgc	gctcactgcc	cgctttccag	tcgggaaacc
3541	tgtcgtgcc	gctgcattaa	tgaatcggcc	aacgcgcggg	gagaggcggt	ttgcgtattg
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3841	agaggtggcg	aaacccgaca	ggactataaa	gataccaggc	gtttccccct	ggaagctccc
3901	tcgtgcgctc	tcctgttccg	accctgcgcg	ttaccggata	cctgtccgcc	tttctccctt
3961	cgggaagcgt	ggcgcctttc	caatgctcac	gctgtaggta	tctcagttcg	gtgtaggctg
4021	ttcgtctcaa	gctgggctgt	gtgcacgaac	cccccgttca	gcccagccgc	tgcgccttat
4081	ccggtaaact	tcgtcttgag	tcacaacccg	taagacacga	cttatcgcca	ctggcagcag
4141	ccactggtaa	caggattagc	agagcgaggt	atgtaggcgg	tgctacagag	ttcttgaagt
4201	ggtggcctaa	ctacggctac	actagaagga	cagtatttgg	tatctgcgct	ctgctgaagc
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4321	gcggtgggtt	ttttgtttgc	aagcagcaga	ttacgcgcag	aaaaaaagga	tctcaagaag
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4441	ttttggtcat	gagattatca	aaaaggatct	tcacctagat	cctttttaa	taaaaaatgaa
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4621	ccgtcgtgta	gataactacg	atacgggagg	gcttaccatc	tggccccagt	gctgcaatga
4681	taccgcgaga	cccacgctca	ccggtccag	atctatcagc	aataaaccag	ccagccggaa
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FIGURE 24C

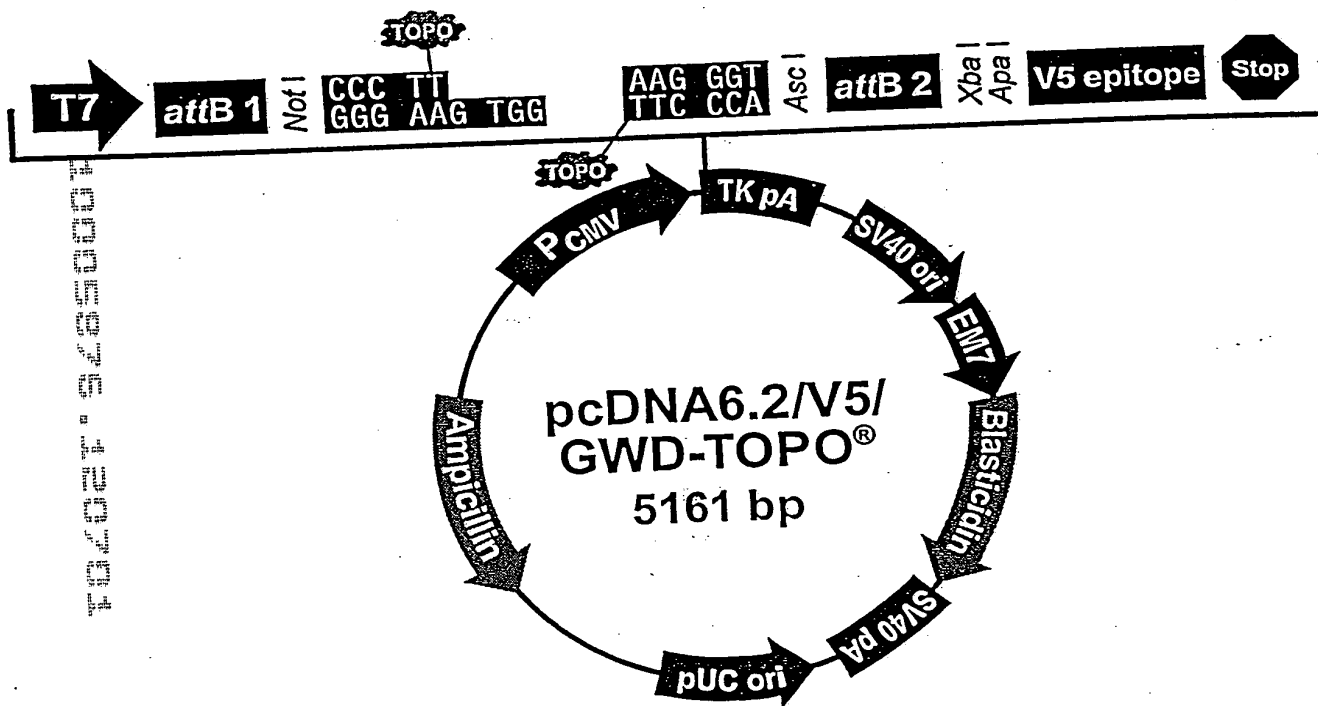


FIGURE 25A

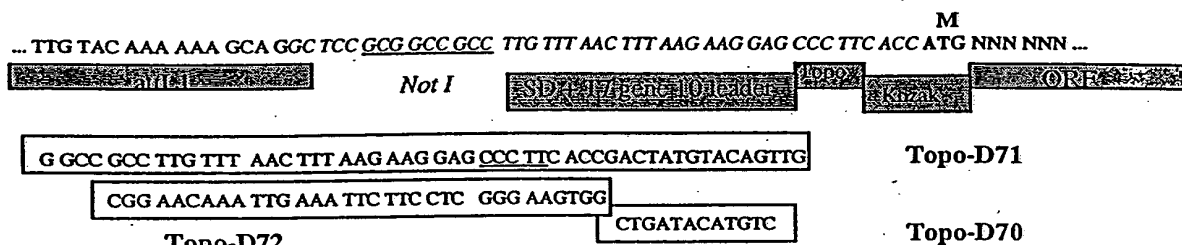
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301 tggagttccg cgttacataa cttacggtaa atggcccgcg tggctgaccg cccaacgacc
361 cccgcccatt gacgtcaata atgacgtatg tcccatagt aacgccaata gggactttcc
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1081 ggtagtaaat gagtttaaac gggggagggt aactgaaaca cggaaggaga caataccgga
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FIGURE 25 B

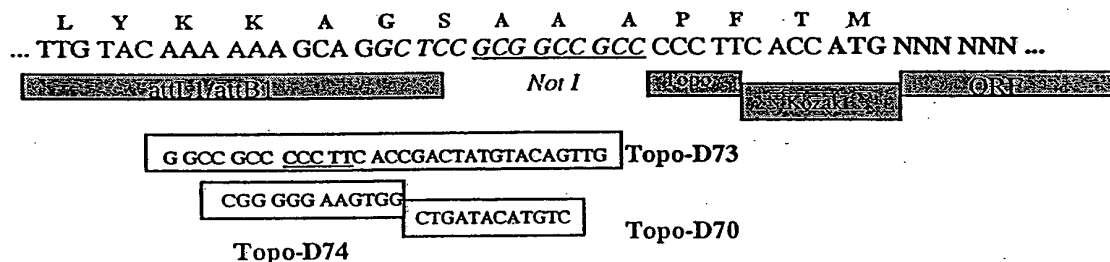
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4261	gataactacg	atacgggagg	gcttaccatc	tggccccagt	gctgcaatga	taccgcgaga
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5101	atttgaatgt	atttagaaaa	ataaacaaat	aggggttccg	cgcacatttc	cccgaagaat
5161	gccacctgac	gtc				

FIGURE 25C

pENTR/SD-dTopo: 5' end



ENTR-dTopo and pcDNAGW-dTopo: 5' end



ENTR/SD-dTopo, pENTR-dTopo, and pcDNAGW-dTopo: 3' end

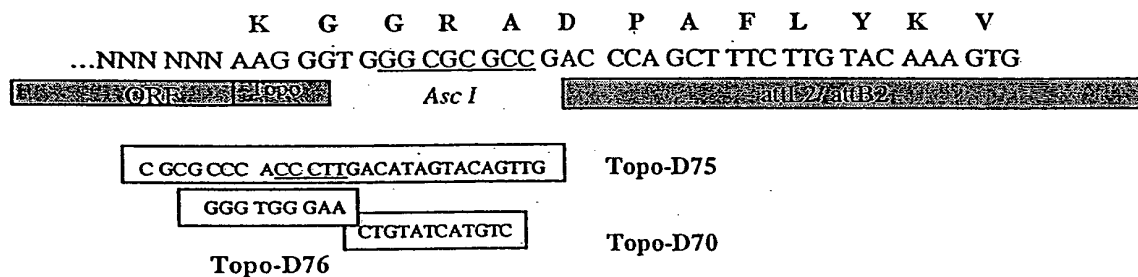


FIGURE 26

1005075 130704
T02021 92090007



FIGURE 27

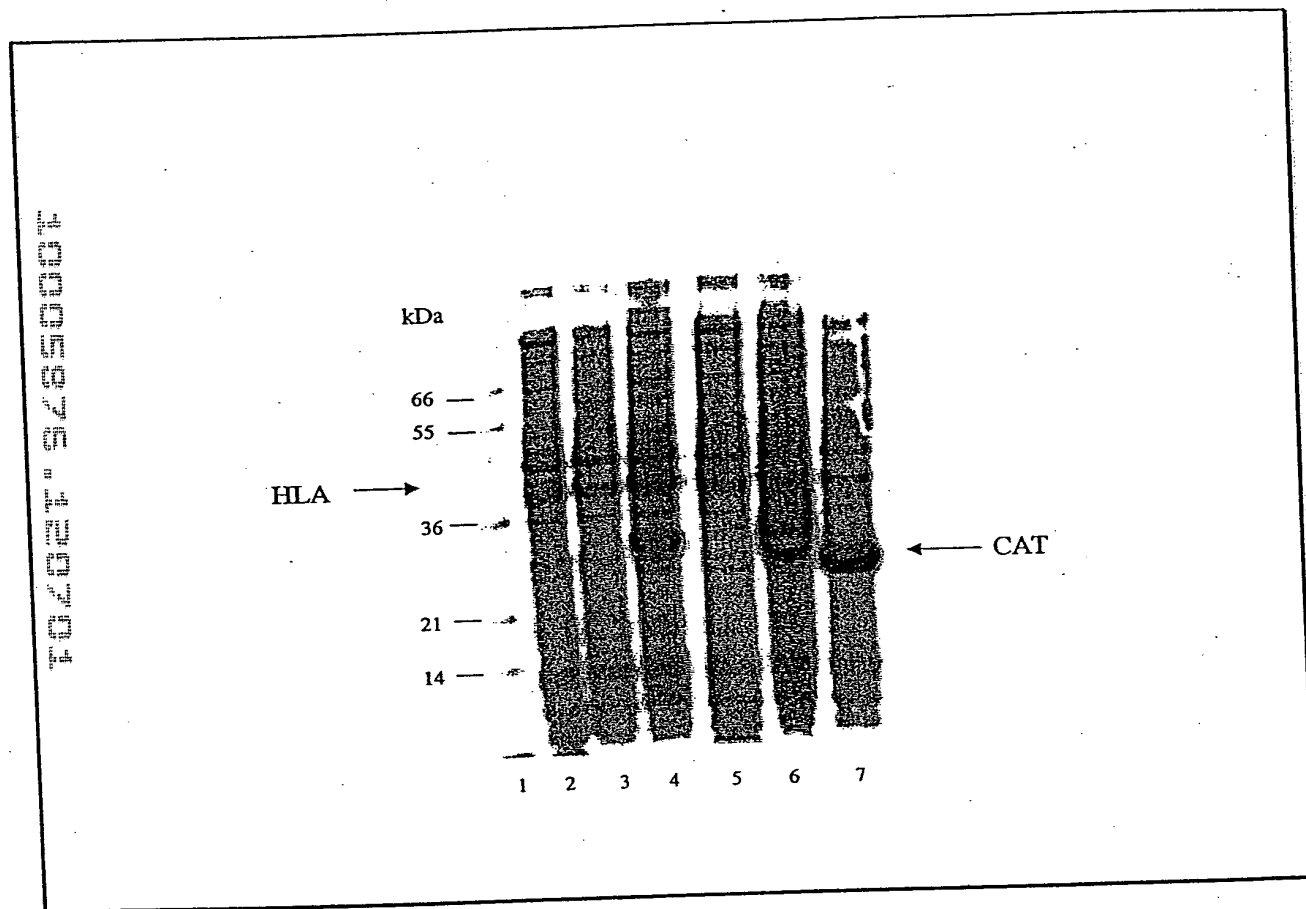


FIGURE 28

1005876 100741

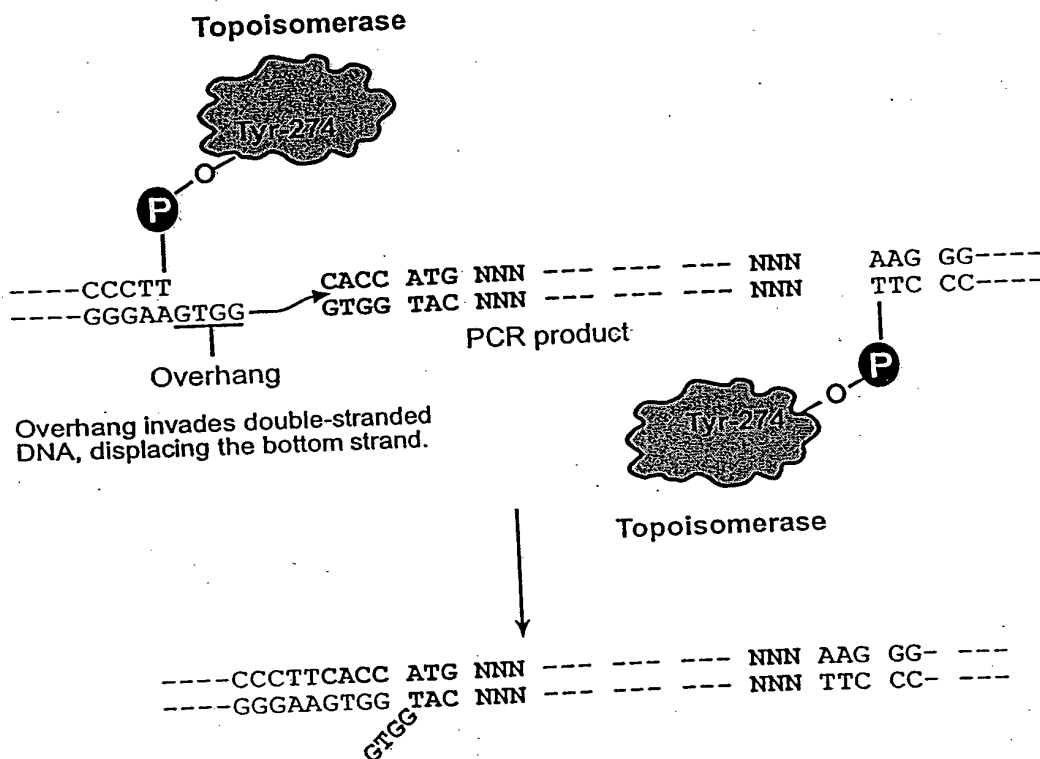
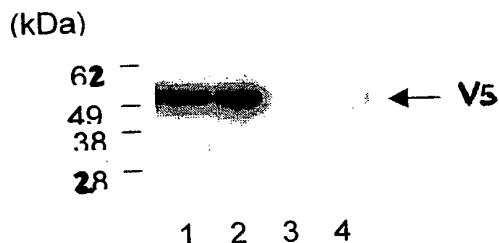


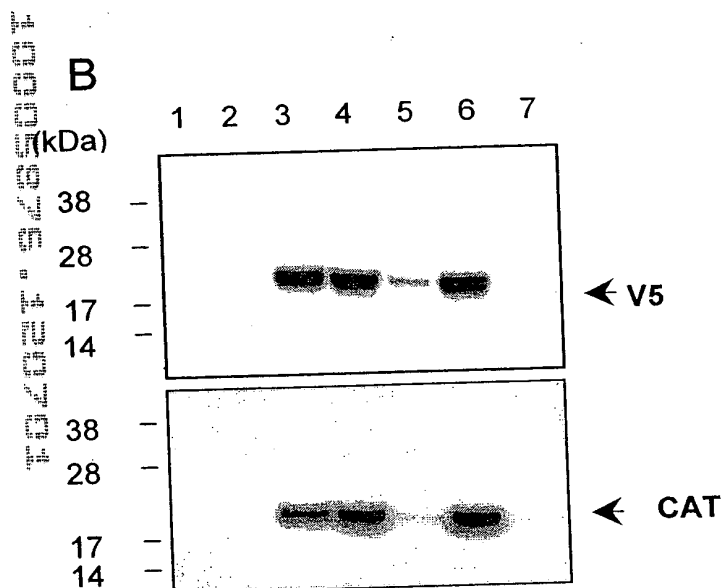
FIGURE 29

A



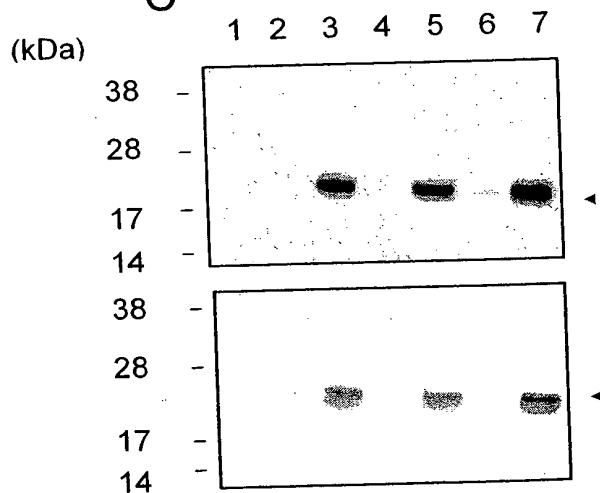
Lane 1: pCMVTetO/CAT/V5TKpA (without secondary PCR)+ Tet
Lane 2: pCMVTetO/CAT/V5TKpA (with secondary PCR)+ Tet
Lane 3: pCMVTetO/CAT/V5TKpA (with secondary PCR) - Tet
Lane 4: pCMVTetO/CAT/V5TKpA (without secondary PCR)- Tet

B



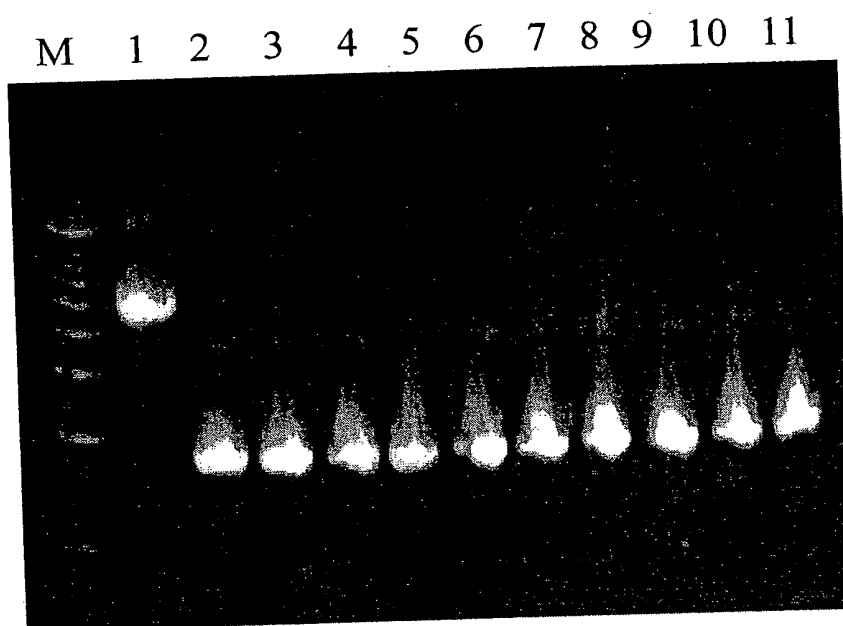
Lane 1: TRex-CHO Cells + Tet
Lane 2: without secondary PCR (with purified CAT) - Tet
Lane 3: without secondary PCR (with purified CAT)+ Tet
Lane 4: without secondary PCR (with unpurified CAT) + Tet
Lane 5: without secondary PCR (with unpurified CAT) - Tet
Lane 6: with secondary PCR + Tet
Lane 7: with secondary PCR -Tet

C



Lane 1: TRex-293 Cells + Tet
Lane 2: without secondary PCR (with purified CAT) - Tet
Lane 3: without secondary PCR (with purified CAT) + Tet
Lane 4: without secondary PCR (with unpurified CAT) - Tet
Lane 5: without secondary PCR (with unpurified CAT) + Tet
Lane 6: with secondary PCR - Tet
Lane 7: with secondary PCR + Tet

FIG. 30



Lane1: negative control; lanes 2-11: test clones; M: 500 bp marker

FIG. 31.

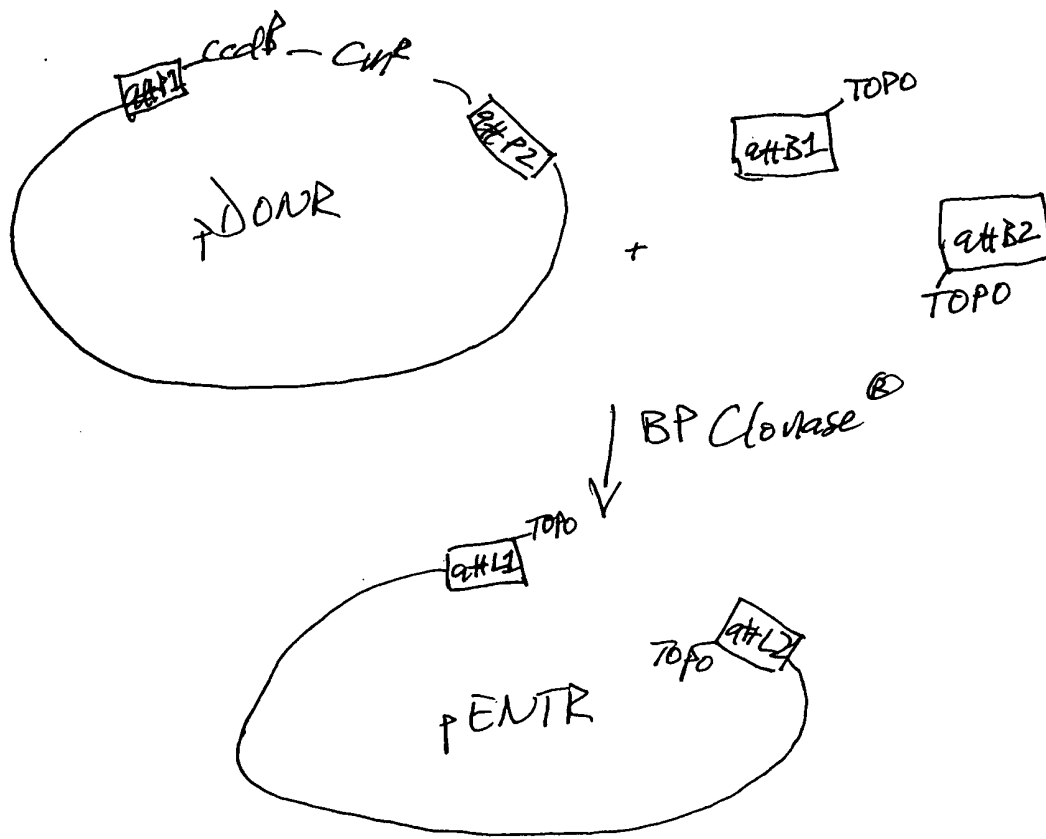
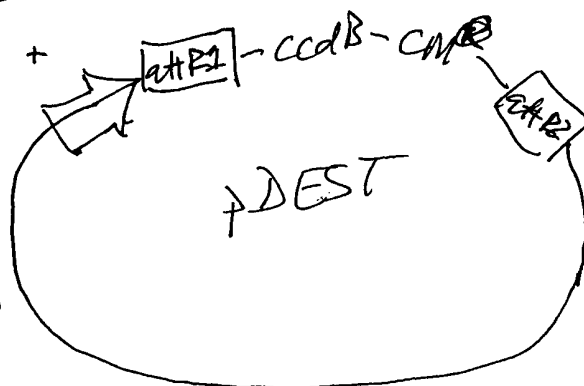
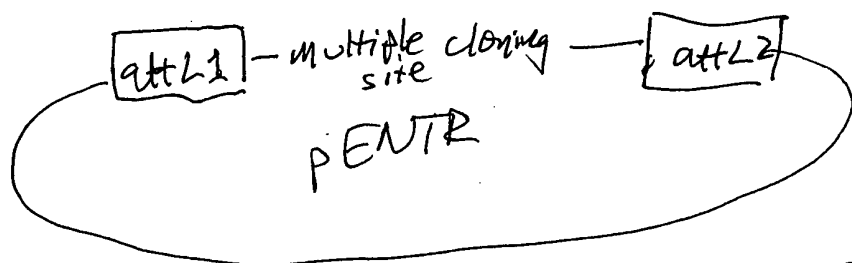


FIGURE 32



✓ ✓
Clonase®



1. Cut with restriction enzymes;
2. Adapt with TOPO adapters;
3. Charge with TOPOLIGASE.
4. Purify

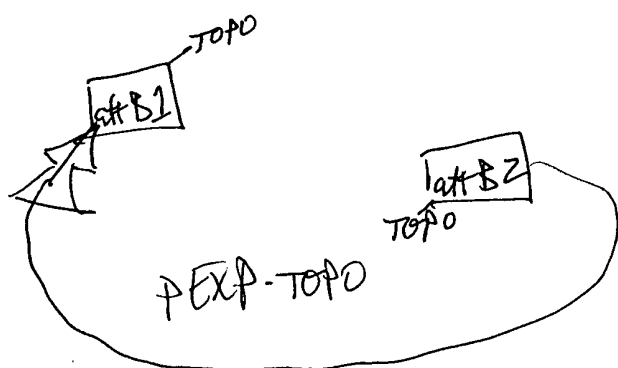


FIGURE 33

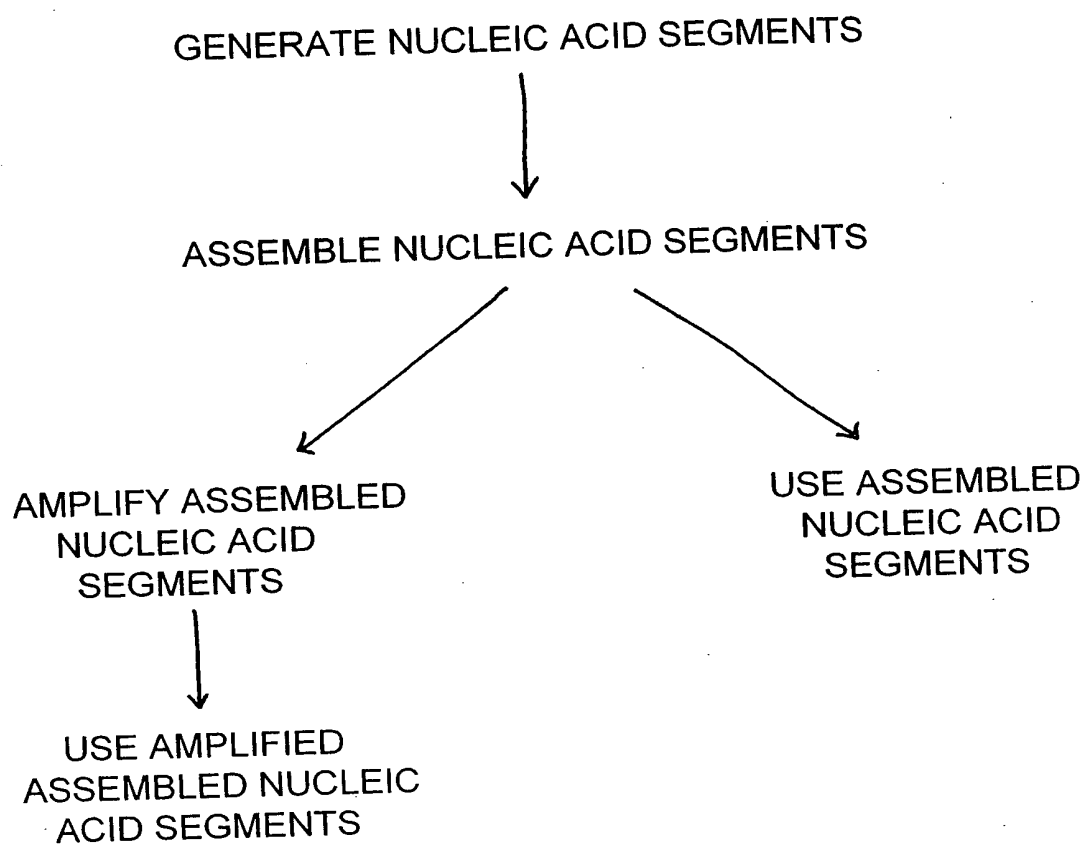


FIG. 34

10005076-120701

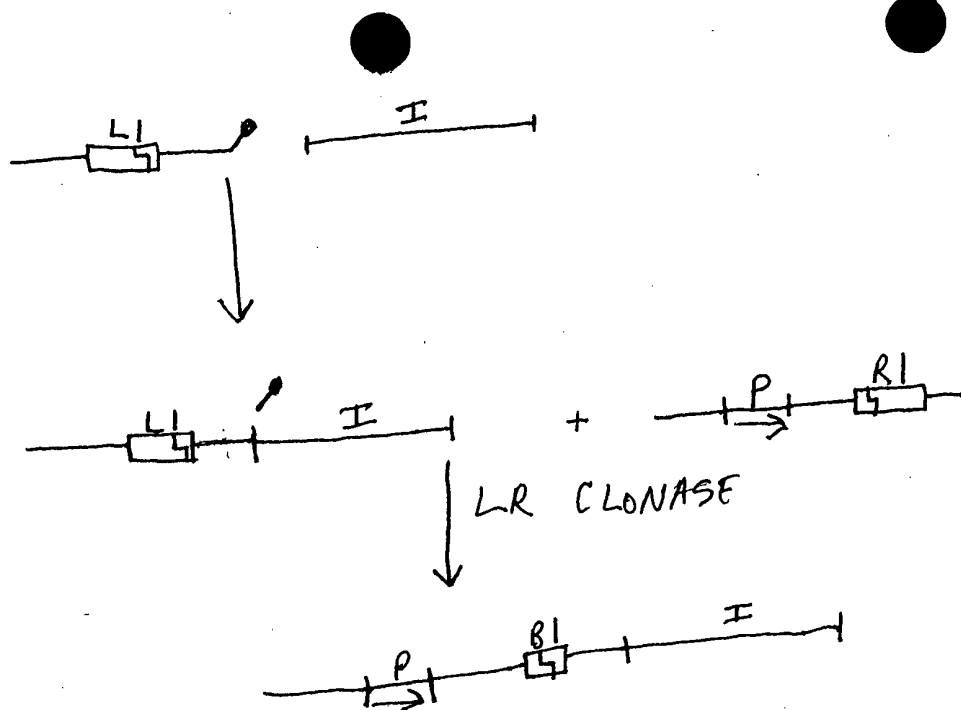


FIGURE 35

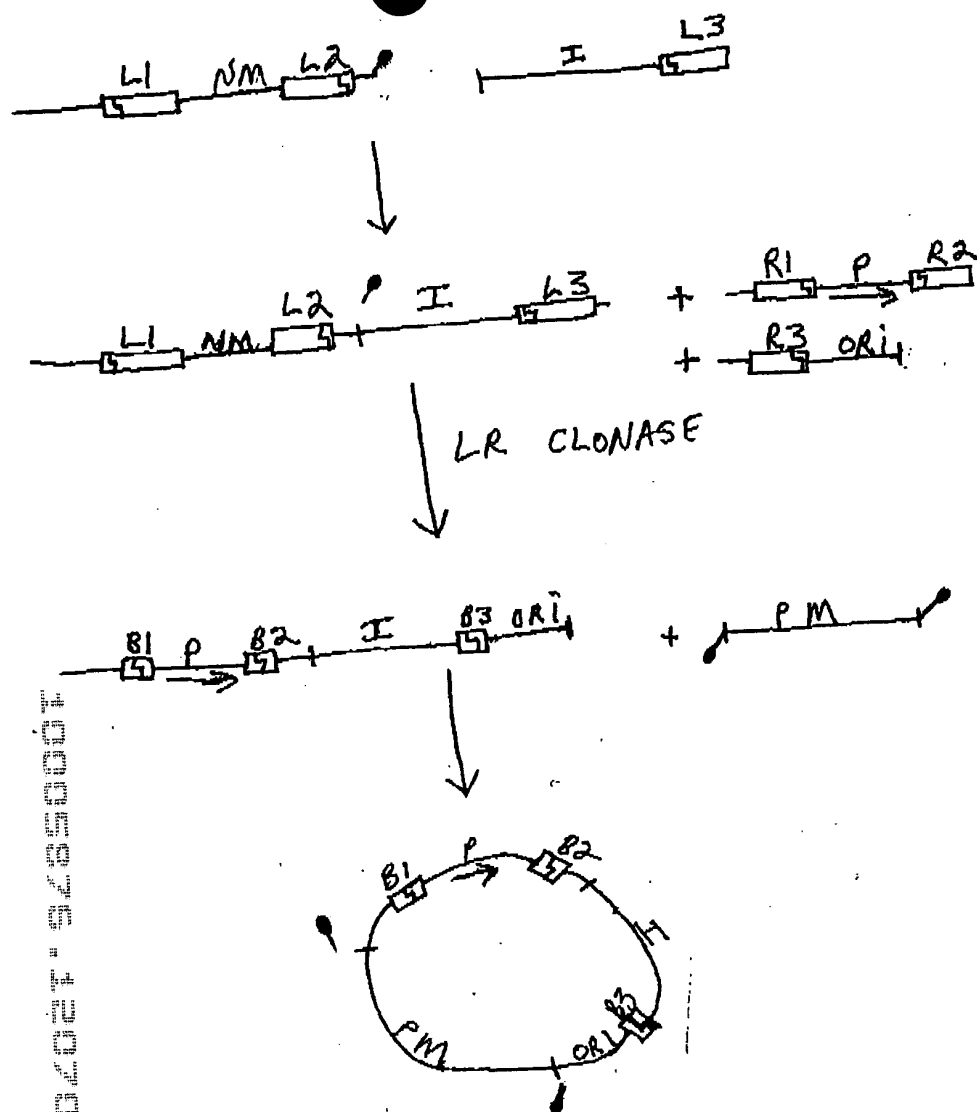


FIGURE 36

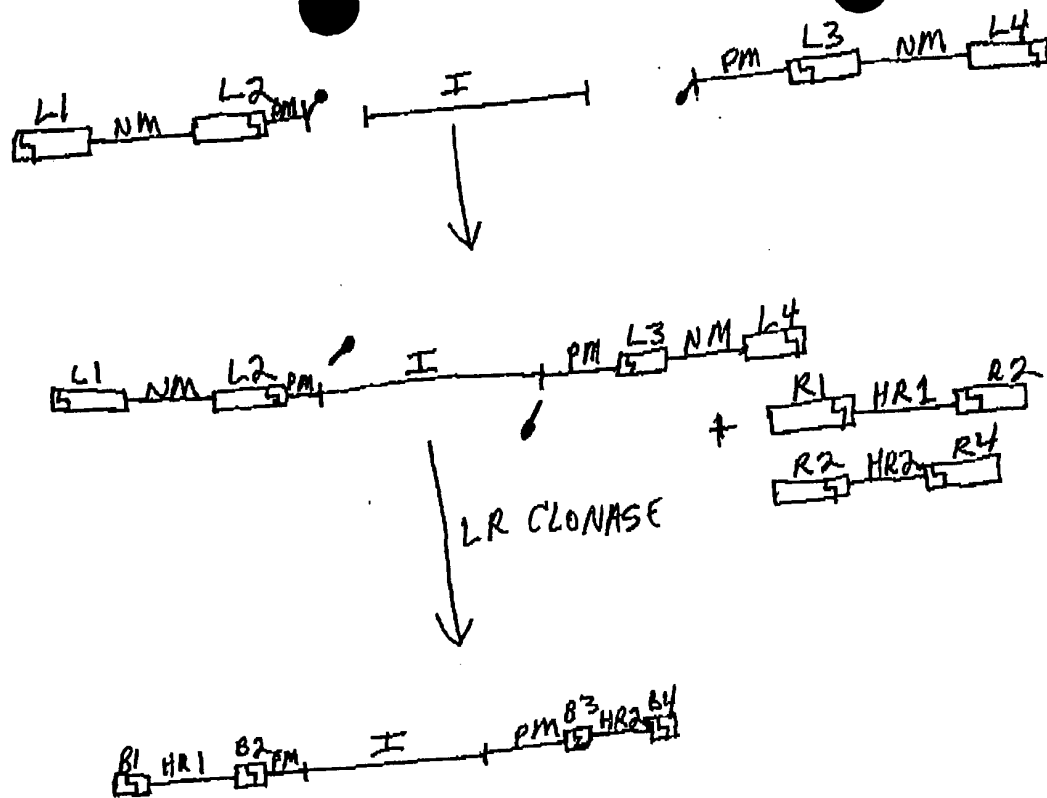


FIGURE 37

1005675-10000

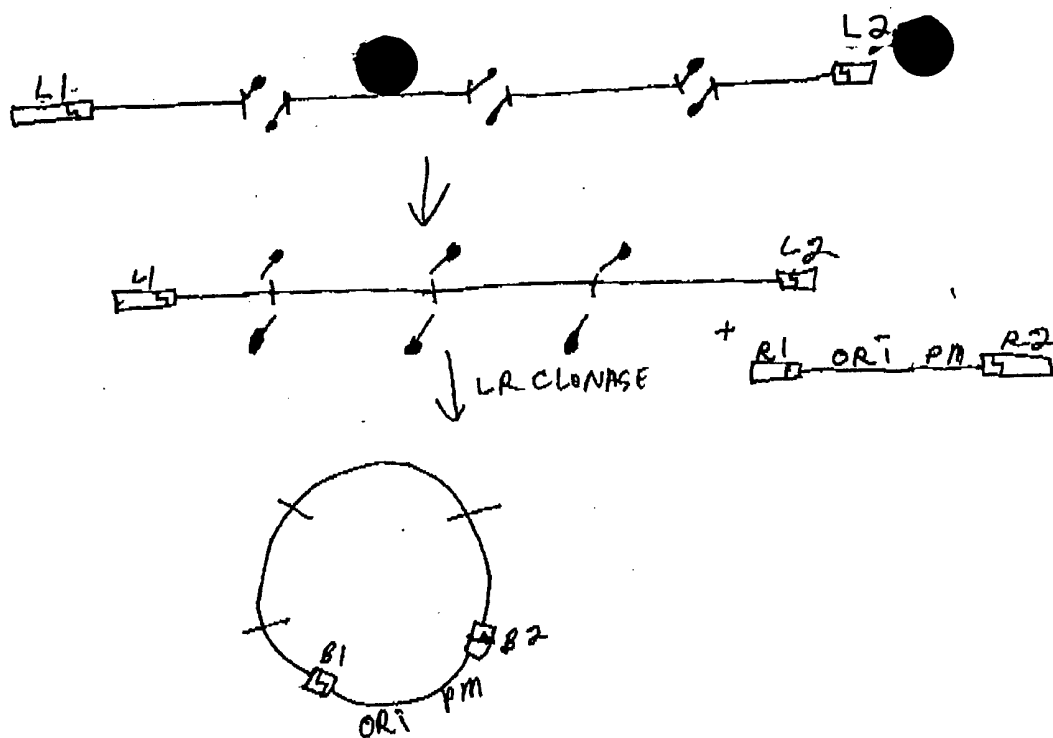


FIGURE 38

10055036 120704

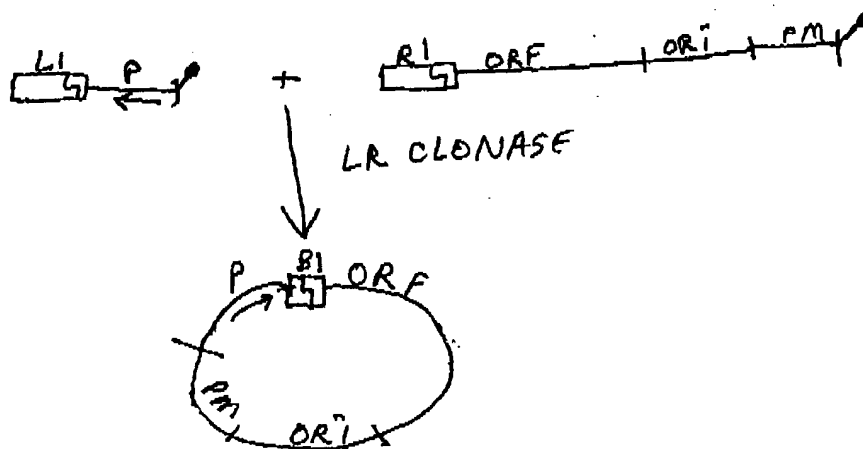


FIGURE 39

1005875 120794

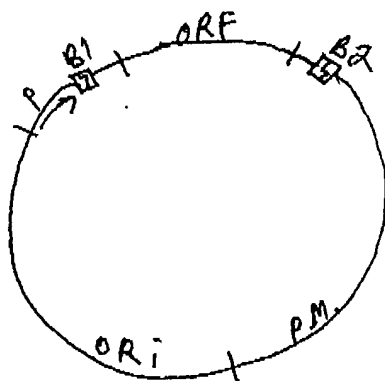
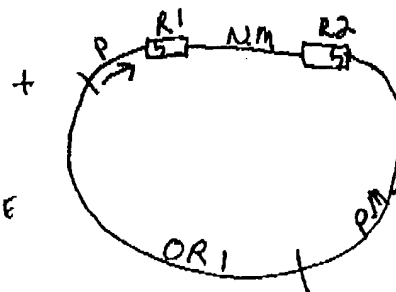
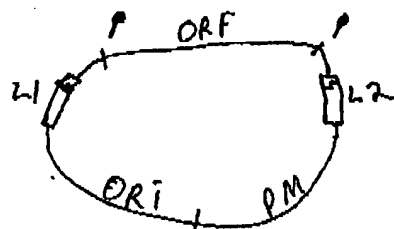
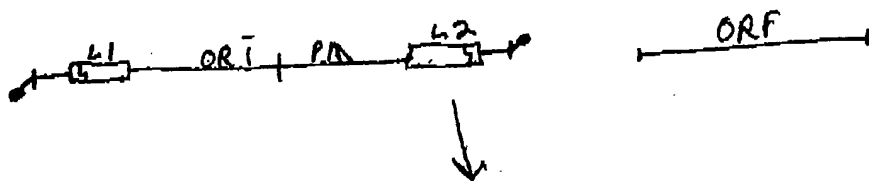


FIGURE 40

10005076.120704